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13. ABSTRACT ***(Maximum 200 Words)***

This project was designed to characterise the sources of phenotypic variability in neurofibromatosis 1 (NF1) by a combination of clinical, statistical, epidemiological, and molecular genetic methods. We have analysed associations found in clinical and genetic data from the NNFF International Database and two other databases with logistic regression and generalized estimating equations. We then extended these methods to use multivariate probit models on familial data. These are novel statistical techniques that have not been used in this way before and yielded interesting results about the sources of phenotypic variability. We were able to determine that the presence of some features of NF1 are more influenced by variability in the *NF1* allele, others by the normal *NF1* allele and still others by unlinked modifying genes. We have set up, and tested the screening protocol to identify the constitutional mutations of NF1 and have obtained a number of blood samples. We have results for one of our phenotypic subgroups and are completing the analysis at the present time.

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INTRODUCTION

The natural history of neurofibromatosis 1 (NF1) is incompletely understood. It exhibits extreme clinical variability and is progressive over the course of an affected individual's life. However, the rate of progression and the occurrence of serious complications vary greatly between different patients of the same age, different affected members of a single family, and even a single affected individual at different times in life. This variability and concomitant uncertainty greatly increases the burden for affected families.

The purpose of this project is to characterize the sources of phenotypic variability in NF1. The studies were performed on the world's largest NF clinical databases, together including over 5000 individuals, and used a variety of statistical and genetic epidemiological methods. Our goal was to determine if a set of clinical phenotypes exists for which allelic differences at the *NF1* locus appear to be important determinants of phenotype. The molecular component of this study (undertaken by Dr. D. Viskochil, University of Utah) was to develop a molecular screening process to enable us to identify the nature of the mutations within these putative phenotypes.

BODY

Data used in these studies:

Most of the initial analyses (tasks 1-4) were initiated using data from the National Neurofibromatosis Foundation International Database (NFDB). At the beginning of this project, this database contained information on about 3000 individuals with NF1. The number continued to increase as data were added over the course of the study. For verification of our findings, to increase the numbers of families with multiple affected members available for analysis, and for extension into population-based data or more specialized analyses, we established collaborations with Dr. Vincent Riccardi and Dr. Gareth Evans. Dr. Riccardi's data, from the NF Institute, (NFID) consists of over 1000 patients, all examined by him personally. We devoted substantial effort to converting Dr. Vincent Riccardi's NF Institute Database from paper files to a fully computerized form for use in this project. Dr. Evans' data (MANF1) consist of clinical information on about 500 individuals, representing ascertainment of approximately 2/3 of the expected NF1-affected individuals in NW England. There is no overlap between the three databases.

Technical objectives 1 and 2: Identification of associations between features of NF1.

Task 1: Identify associations of features within probands by screening the 5000 possible pair-wise associations on both databases.

We began our analysis in year 1 by producing two-by-two tables for the approximately 100 features in the National Neurofibromatosis Foundation International Database (NFDB). This

resulted in a total of more than 5000 tables. Our plan was to examine statistically significant associations for covariates such as age, gender and race, then to examine each association for the presence of higher order associations. In order to identify all potential associations, we used *chi-square* calculations with a nominal significance level of p=.05 and odds ratios with 95% confidence intervals that do not overlap 1 to identify potential associations for further study. A more conservative Bonferoni correction for multiple comparisons was not used in order to avoid eliminating small but potentially important associations.

The statistically significant (but not necessarily clinically significant) associations were identified. The analyses were repeated for all three databases and summary odds ratios calculated. These studies are presented in task 3, where the associations were subjected to more rigorous scrutiny in order to identify potential covariates and eliminate spurious associations.

Task 2: Identify associations of NF1 features between related individuals (e.g. parent-child diads) in both databases.

Using methods similar to those in task 1, but between relatives, including parent-child diads and sibship diads, we performed 2 x 2 analyses to look for familial tendencies for the occurrence of certain clinical features. We were able to demonstrate that such relationships existed for features such as optic gliomas, discrete dermal neurofibromas, and Lisch nodules among unselected patients with NF1 [See Szudek et al., 1999A (Appendix)]. Some of these familial associations are of strikingly similar strength in sib-sib and parent-child comparisons. Other associations, such as those for dermal neurofibromas, are apparent in sibs but not in parent-child pairs, raising the possibility of confounding by age. We, therefore, extended these studies with logistic regression analysis and a multivariate probit model, both of which permit adjustment for the effect of age. These analyses are described under task 4.

Task 3: Examine apparent associations (from task 1) in detail for other covariates or confounding factors, using log-linear models for binary traits and stratification for age or other continuous variables.

We began to examine potential associations for covariates in year 1 using methods appropriate for the covariate: For variables with only two possible values (e.g. as gender) or categorical variables (e.g. race or parent of origin), we were able to perform 2 x 2 x n tables (where "n" represents the number of categories of the potential covariate). The same statistical tests of significance were applied as for the 2 x 2 tables. We found consistent phenotypic associations among various clinical features of NF1 using age-stratified 2 x 2 analyses [Baser et al., 1999 (Appendix); Szudek et al., 1999A (Appendix)], but these phenotypic associations were generally neither strong enough nor characteristic enough to establish prognostically important subgroups of NF1 patients. We, therefore, attempted to extend this analysis by using log-linear models. These efforts were unsatisfactory because log-linear models can only control for age by stratification. We found that differential age adjustment by feature was

necessary because each feature of NF1 has a unique relationship with age. Furthermore, stratification requires the establishment of arbitrary age groups that permit only partial adjustment for age.

We therefore undertook a series of logistic regressive analyses. These techniques allow age to be transformed to reflect its unique relationship with each feature. In addition, age can be treated as a continuous variable, which increases precision. Logistic regression also permits the simultaneous analysis of associations of more than two features.

We selected 12 especially important or frequent clinical features of NF1 and treated each feature in turn as if it were a disorder that occurs in a population affected with NF1. Using logistic regression, we asked: What is the relationship of the selected (outcome) feature to the other 11 (explanatory) features? (The use of the terms "outcome" and "explanatory" to describe variables is conventional in logistic regression analysis. We are not suggesting a causal relationship between explanatory and outcome features). The logit (log of the odds) of each of the 12 features was set as the output variable in a different logistic regression model. Age was controlled as a continuous covariate. Univariate analyses were used to screen for potential main effects. The importance of each of the major explanatory variables was simultaneously assessed in a logistic regression model. Significant terms were used to refit the model, and interaction terms among the explanatory variables were considered.

Fitted logistic regression models can be expected to perform well on the data set used to develop them. Therefore, we developed our models in a random subset of data from half of the 2,797 NF1 probands available to us from the NNFF International Database and tested the best fitting models on data from the subset of probands that was was initially excluded. Forward selection by maximum likelihood estimation was used to identify and quantify significant explanatory features in the developmental subset. Hosmer and Lemeshow goodness-of-fit was calculated for each model. Parameter estimates and goodness-of-fit were then calculated independently in the originally excluded subset, and the results of were compared (Table 1). Models that provided a good fit and had similar parameter estimates in both subsets were considered to be accurate.

Logistic regressive models allow the associations between two or more features to be described as odds ratios. Table 2 shows the odds ratios for the more reliable associations found in the analysis reported in Table 1. Figure 1 shows a proposed grouping schematic of NF1 features based on the odds ratios in table 2. Our findings demonstrate that some NF1 patients are far more likely than others to have certain common features of the disorder and that the presence of some features makes the presence of other features more likely. For more detail on the methods used here, please see the submitted manuscript, (Szudek et al, Logistic Regressive Models of NF1) included in the Appendix

Table 1: Summary of parameter estimates in logistic regressive models of NF1 clinical features.

Output Feature	Explanatory Feature	Initial (Developmental) Subset		Second (Validation) Subset	
		Parameter Estimate	Goodness-of-fit	Parameter Validation	Goodness-of-fit
Freckling	Lisch nodules Neoplasms Female gender	0.2180 0.7484 0.3804	p=0.8753	0.3067 0.6069 0.2022	p=0.7752
Discrete NFs*	Plexiform NFs Lisch nodules	1.2092 0.4642	p=0.8083	0.7870 0.2755	p=0.4085
Plexiform NFs	Discrete NFs Learning disability Scoliosis	1.1301 0.2092 0.6375	p=0.8539	1.1634 0.1556 0.2176	p=0.4064
Lisch nodules	Freckling Discrete NFs Neoplasms	0.3084 0.3280 1.5306	p=0.9235	0.2923 0.5809 0.5445	p=0.5891
Optic glioma	Plexiform NFs Macrocephaly Neoplasms	0.6634 0.4788 1.9652	p=0.4972	0.4987 0.4805 1.9080	p=0.8989
Learning disability	Plexiform NFs Seizures Short stature Male gender	0.4616 0.7962 0.3352 0.3653	p=0.3024	-0.1108 0.0555 0.5622 0.4890	p=0.4897
Seizures	Lisch nodules Learning disability Male gender	-0.5305 0.9719 0.4567	p=0.7901	-0.2391 0.6174 -0.0041	p=0.7381
Pseud-arthrosis	Freckling Discrete NFs Neoplasms	-1.0351 -0.8735 0.7462	p=0.8419	-0.5078 -0.4674 0.3132	p=0.9171
Scoliosis	Plexiform NFs Learning disability	0.6216 0.5504	p=0.0316	-0.6558 0.0290	p=0.0403
Macro-cephaly	Optic glioma Short stature	0.4643 -0.7692	p=0.2853	0.5129 -1.3933	p=0.3710
Short stature	Learning disability Macrocephaly	0.4082 -0.9804	p=0.9960	0.5694 -1.8413	p=0.6211
Neoplasms	Lisch nodules Optic glioma	0.9250 1.8711	p=0.6073	0.4816 1.5751	p=0.0855

* NFs = neurofibromas

Table 2: Summary of associations using logistic regressive models of NF1 clinical features. Results are given as odds ratios with 95% confidence intervals.

Feature	Associated Features	Odds Ratio (95% C.I.)	
Freckling	Lisch nodules	1.2	(0.8-1.9)
	Neoplasms	2.1	(0.7-6.0)
	Male gender	1.4	(1.1-2.1)
	All three	*3.8*	*(1.2-12)*
Discrete NFs*	Plexiform NFs	3.4	(2.2-4.9)
	Lisch nodules	1.6	(1.1-2.2)
	Both	*5.3*	*(3.3-8.7)*
Plexiform NFs	Discrete NFs	3.1	(2.1-4.6)
	Learning disability	1.2	(0.9-1.7)
	Scoliosis	1.9	(1.3-2.7)
	All three	*7.2*	*(4.0-13)*
Lisch Nodules	Freckling	1.4	(0.9-2.0)
	Discrete NFs	1.4	(1.1-1.9)
	Neoplasms	4.6	(2.1-10)
	All three	*8.7*	*(3.5-22)*
Optic glioma	Plexiform NFs	1.9	(0.9-4.0)
	Macrocephaly	1.6	(0.9-2.9)
	Neoplasms	7.1	(2.8-18)
	All three	*22*	*(5.7-87)*
Pseudarthrosis	No Freckling	2.8	(1.6-4.9)
	No Discrete NFs	2.4	(1.4-4.2)
	Neoplasms	2.1	(0.9-5.2)
	All three	*6.7*	*(3.2-14)*
Neoplasms	Lisch nodules	2.5	(1.1-6.0)
	Optic glioma	6.4	(3.2-13)
	Both	*16.4*	*(5.5-48)*

* NFs = neurofibromas

After identifying possible associations in the NFDB, we performed the same tests in two other databases, using the data of Dr. Vincent Riccardi of the NF Institute (NFID) and the Manchester data of Dr. Gareth Evans (MANF1). Summary Odds ratios were calculated. These data are presented in the Appendix in the paper by Szudek et al, 2000a.

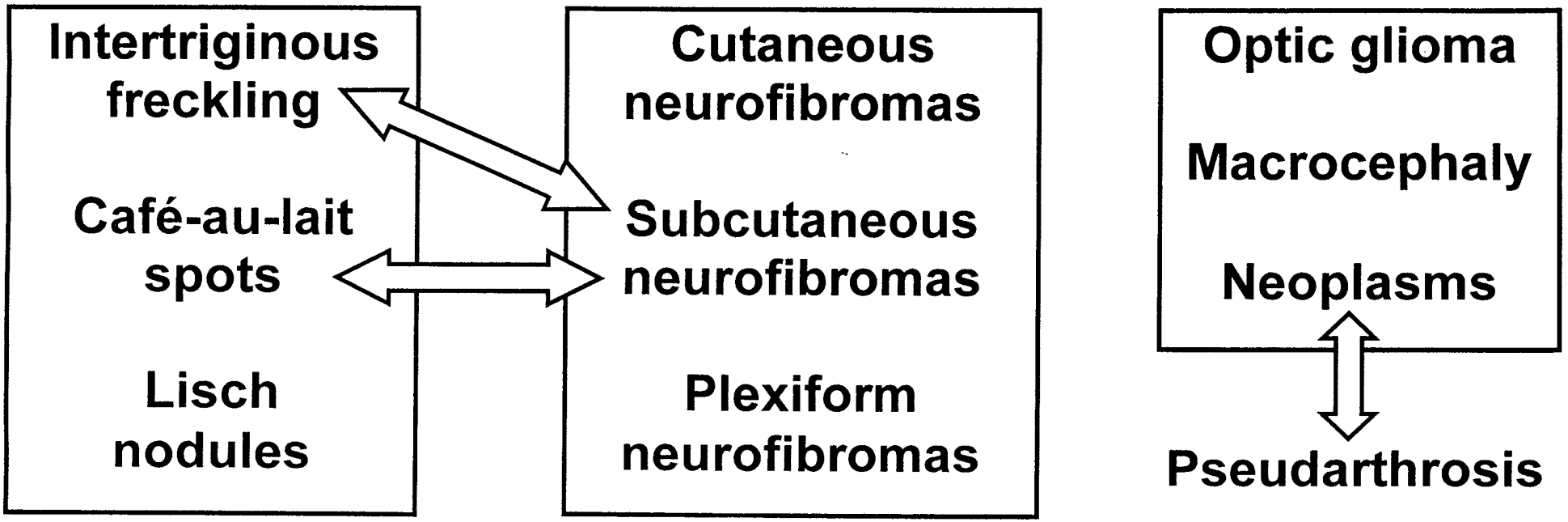


Figure 1: Proposed grouping of NF1 features, based on the odds ratios in Table 2. Features enclosed by a box or connected by an arrow are important variables in each other's models.

An important possible explanation for at least some of the associations seen in the research outlined in task 1 and above in task 3 could be the influence of local factors. Assessment of this possibility was not part of our original research plan but deserved consideration. For example, we found that individuals with diffuse plexiform neurofibromas are more likely also to have cutaneous neurofibromas. Virtually all diffuse plexiform neurofibromas are of congenital origin thus it is possible that they influence the development of cutaneous neurofibromas. We tested the hypothesis that the development of these lesions might be influenced by such local factors by using the detailed dermatological data in the NF Institute Database. For each patient, the presence of 1 or more café-au-lait macule, diffuse plexiform neurofibroma , or cutaneous neurofibroma was recorded for each of ten divisions of the body surface (for a more detailed description of the method, please see the submitted paper, Palmer et al, in the Appendix). We looked for associations between the distributions of café-au-lait macules, cutaneous neurofibromas, and plexiform neurofibromas in10 body segments of 547 patients. No association was observed between the occurrence of cutaneous and diffuse plexiform neurofibromas in the same body segment (OR=1.2, 95% CI=0.8-1.8). Similarly, there was no association between the presence of café-au-lait macules and either cutaneous or diffuse plexiform neurofibromas within a single body segment (OR=1.4, 95% CI=0.9-2.0 for café-au-lait macules with cutaneous neurofibromas, and OR=1.2, CI=0.7-2.1 for café-au-lait macules with plexiform neurofibromas).

Our in-depth analysis of the many features of NF1 revealed a number of interesting observations important to our understanding of the variability of the phenotype of this condition. One of the initial variables examined in tasks 1 and 3 was that of cardiovascular malformations. We found that the incidence of several cardiac malformations was greatly increased over the general population. In particular, the incidence of pulmonic stenosis was about 6 times greater than in the general population. We were, however, unable to find any statistically significant associations of other NF1 features with any of the cardiovascular abnormalities we tested. We did find an apparent association between renal artery stenosis and aortic coarctation. For more a more detailed discussion of these findings, please see Lin et al, 2000, in the Appendix.

Our association studies and studies of covariates included gender. The majority of features of NF1 are equally frequent in males and females. The pseudarthrosis spectrum (long bone bowing through frank fracture) is one notable exception: We found a male predominance in both incidence and severity. For more detail on this study, please see Stevenson et al, 1999, in the Appendix.

We noted the expected finding of an overall association between scoliosis and the presence of dysplastic vertebrae. Interestingly, however, closer examination revealed that some scoliosis was associated with vertebral scalloping, whereas other scoliosis was not. In order to quantify this finding, we developed a physical measure of vertebral scalloping and applied it to a series of radiographs of patients with NF1 and scoliosis. We found a bimodal distribution of vertebral scalloping such that some patients with NF1 and scoliosis had vertebral scalloping whereas other patients with NF1 and scoliosis had vertebrae that were similar in shape to a matched sample of people with scoliosis but without NF1. This study indicated that there is a subset of NF1 patients who have increased vertebral scalloping. Please see the Appendix for a pre-print of this research by Kwok et al, 2001.

Task 4: Perform detailed examination of apparent familial associations identified in task 2.

Our 2 x 2 analyses (task 2) demonstrate familial tendencies for the occurrence of clinical features such as optic gliomas, discrete dermal neurofibromas, and Lisch nodules among unselected patients with NF1 [See Szudek et al., 1999A (Appendix)]. Some of these familial associations are of strikingly similar strength in sib-sib and parent-child comparisons. Other associations, such as those for dermal neurofibromas, are apparent in sibs but not in parent-child pairs, raising the possibility of confounding by age. We, therefore, extended these studies with logistic regression analysis and a multivariate probit model, both of which permit adjustment for the effect of age.

The logistic regression analysis done for features in individual patients (Table 2) was extended to examine associations of common features among relatives. The method of Liang and Beaty (1991; Liang et al., 1992) is based on a logistic regression model that incorporates effects of individual covariates while measuring familial aggregation of risk as the odds ratios between classes of relatives. We used this method with the software "Generalized Estimating Equations 2" (GEE2), developed by Liang and Beaty, to incorporate what we had learned regarding associations between features in probands and simultaneously measure familial aggregation of risk. The associations we found between relatives are summarized in Table 3 as odds ratios with adjustment for the confounding effect of age.

Table 3: Summary of familial associations of NF1 clinical features using logistic regression. 171 NF1 patients and 214 of their affected sibs pairs and 211 affected parents and 289 affected children from the NNFF International Database were analysed using the method and GEE2 software described by Liang and Beaty (1991).

Feature	Odds-ratio (95% C.I.)			
	Sib-Sib		Parent-Child	
Freckling	4.9	(1.8-13)	2.4	(0.7-8.6)
Discrete NFs	6.0	(2.4-15)	4.3	(0.1-170)
Plexiform NFs	1.2	(0.4-3.8)	1.7	(0.7-4.2)
Lisch nodules	13.0	(3.7-44)	3.9	(1.1-15)
Learning disability	3.6	(1.8-7.6)	1.0	(0.5-1.9)
Scoliosis	1.8	(0.5-6.5)	1.0	(0.3-3.0)
Macrocephaly	5.3	(1.5-19)	3.5	(1.0-13)
Short stature	4.0	(1.1-14)	5.3	(1.7-16)
Neoplasms	21.8	(1.5-310)	20.9	(5.3-82)

Again, after identifying possible associations in the NFDB, we performed the same tests in the NFID and MANF1 data and calculated summary odds ratios. (Please see Appendix, Szudek et al, 2000A).

As explained in task 3, above, we were able to use the detailed dermatological data of the NF Institute to look for local associations between some of the skin findings in NF1. We used the same data to examine the influence of familial factors in more detail. For this analysis, patients were stratified into 5 year age increments, the total number of body segments affected with cutaneous neurofibromas was calculated, and patients ranked by the number of affected segments. We then used random effects models to obtain maxiumum likelihood estimates and confidence intervals for intrafamilial correlations for decile. Café-au-lait macules and plexiform neurofibromas were also analysed in the same manner. (For a more detailed description of the method, please see the submitted paper, Palmer et al, in the Appendix). We found significant intrafamilial correlations in the number of body segments affected by each of the clinical features. The correlation among relatives for cutaneous neurofibromas was 0.37 (95% CI=0.15-0.55), for plexiform neurofibromas was 0.35 (95% CI=0.15-0.57), and for café-au-lait macules was 0.45 (95% CI=0.18-0.71). These data present a different approach to looking for associations and are consistent with the other evidence we have amassed relating these features. These associations therefore suggest a genetic influence on the severity of the NF1 lesions and are consistent with multiple factors being involved in the pathogenesis of both plexiform and cutaneous neurofibromas as well as of café-au-lait macules.

Technical objective 5: Determine the contribution of genetic and non-genetic factors to the presence or absence of certain NF1 traits.

Task 5: For traits showing familial aggregation (from task 2), partition variance by using various techniques including logistic regressive modelling and multivariate normal methods.

In order to extend the analyses in task 4, above, we wished to include data available for more distant degrees of relationship. This enabled us to derive more information about the genetic sources of any similarities in features within families. The GEE2 program does not allow for complete intra-familial relationship classifications. We therefore used newly developed programs for multivariate probit analysis (MPROBIT) and mutivariate normal analysis (MVNFAM) (Joe, 2000) to extend the analyses in Task 4. Using these methods, we were able to measure associations of NF1 features among affected sib-sib pairs, children and their mothers and fathers, and second degree relatives such as proband-aunt/uncle/grandparent. Simultaneously, we were able to take other clinical features and age into account and adjust for the non-independence of affected relatives. We used binary data from the NFDB and ordinal data, where available, from the NF Institute, from sib-sib, parent-child, 2nd degree relative pairs and examined 12 clinical features identified as potentially important in our previous studies. (For more details on the methods, please see Appendix for the manuscript submitted for publication by Szudek et al, Analysis of Intra-Familial Phenotypic Variation in NF1.). Table 4 and Figure 2 summarize these results for all relatives taken as a whole from the NFDB. Statistically significant positive intrafamilial correlations or latent correlations were observed for

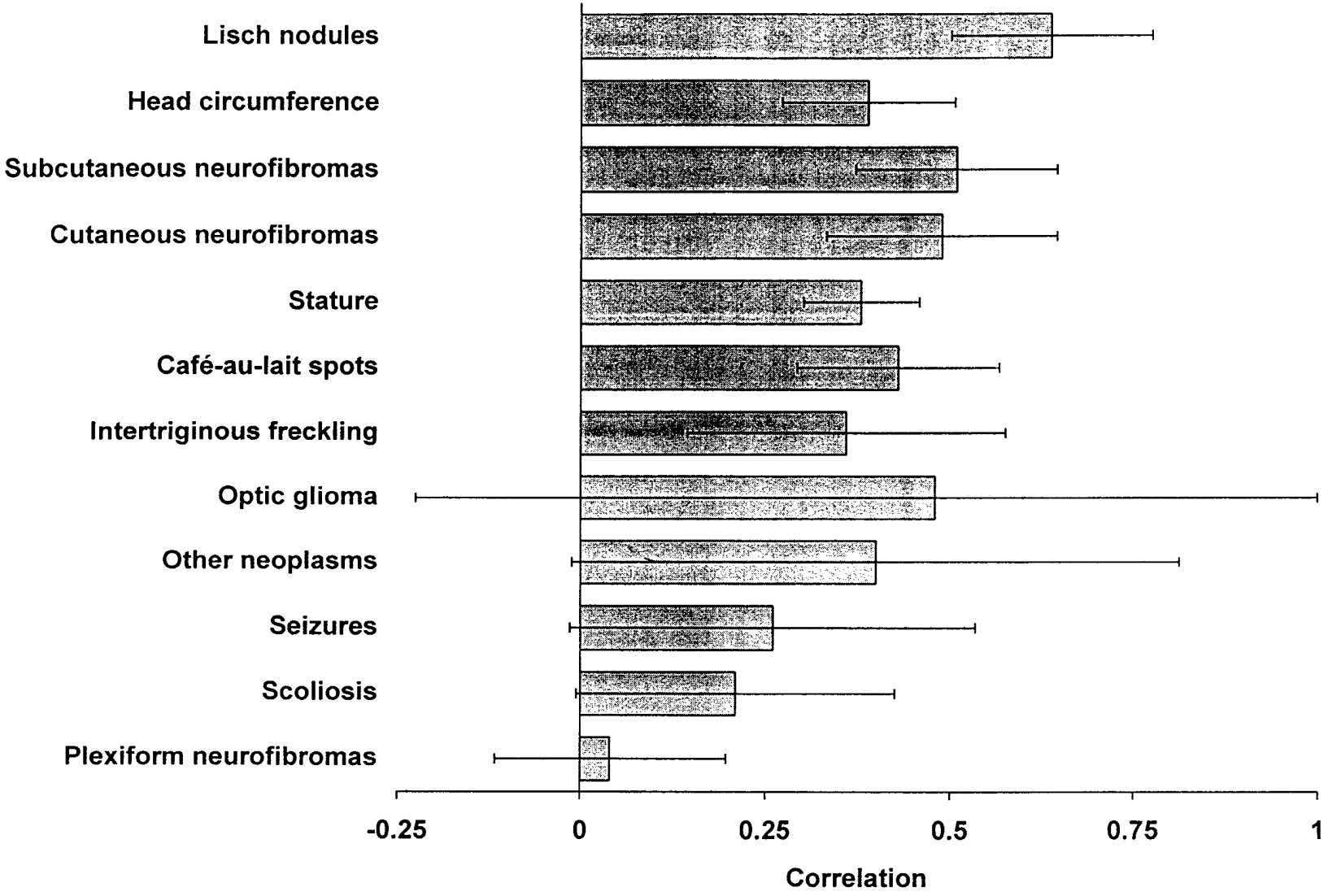
Table 4. Summary of regressions in multivariate models for 12 clinical NF1 features. The 1st column lists the 12 modelled features. The 2nd–4th columns show the covariates and their regression parameter estimates (β) with standard errors (SE) used in each model. β_0 is the intercept in the model equation. Each regression accounts for covariates such as related features, interactions between related features, age and gender. Interactions are depicted by features separated by an "*" and their values equal the product of the two interacting features.

Modelled Feature	**Intercept and Covariates**	β	**SE**
Lisch nodules	β_0	.65	(.08)
	Age	-3.55	(.32)
	Male gender	-.01	(.08)
	Café-au-lait spots	.23	(.15)
	Cutaneous neurofibromas	.44	(.20)
	Café-au-lait spots * Cutaneous neurofibromas	-.09	(.22)
Café-au-lait spots	β_0	.28	(.14)
	Age	-.66	(.25)
	Male gender	.03	(.09)
	Intertriginous freckling	.51	(.12)
	Subcutaneous neurofibromas	-.41	(.26)
	Intertriginous freckling* Subcutaneous neurofibromas	.61	(.28)
Head circumference	β_0	-.99	(.10)
	Age	.62	(.21)
	Male gender	-.09	(.31)
	Lisch nodules	-.06	(.36)
	Optic glioma	.56	(.44)
	Stature	.34	(.04)
	Neoplasms	.10	(.75)
Cutaneous neurofibromas	β_0	-1.62	(.11)
	Age	-5.56	(.36)
	Male gender	.01	(.10)
	Subcutaneous neurofibromas	.62	(.11)
	Plexiform neurofibromas	.36	(.12)
Stature	β_0	-.62	(.09)
	Age	-.82	(.31)
	Male gender	-.03	(.09)
	Head circumference	.04	(.01)
Optic glioma	β_0	-1.02	(.13)
	Age	.72	(.57)
	Male gender	.06	(.17)
	Plexiform neurofibromas	.01	(.37)
	Head circumference	.19	(.07)
	Neoplasms	.55	(.49)

Table 4 (continued)

Modelled Feature	Intercept and Covariates	β	SE
Subcutaneous neurofibromas	β_0	-1.72	(.12)
	Age	-3.78	(.35)
	Male gender	-.04	(.08)
	Café-au-lait spots	.43	(.11)
	Cutaneous neurofibromas	.73	(.13)
	Plexiform neurofibromas	.52	(.17)
	Intertriginous freckling * Plexiform neurofibromas	-.24	(.23)
Intertriginous freckling	β_0	.49	(.15)
	Age	-1.58	(.30)
	Male gender	-.23	(.12)
	Café-au-lait spots	.52	(.14)
	Subcutaneous neurofibromas	-.18	(.27)
	Lisch nodules	.55	(.14)
	Café-au-lait spots * Subcutaneous neurofibromas	.62	(.33)
Seizures	β_0	-1.43	(.11)
	Age	-.88	(.65)
	Male gender	-.04	(.15)
Plexiform neurofibromas	β_0	-1.11	(.11)
	Age	-.88	(.38)
	Male gender	.07	(.09)
	Subcutaneous neurofibromas	.46	(.16)
	Cutaneous neurofibromas	.37	(.14)
	Subcutaneous * Cutaneous neurofibromas	-.21	(.22)
Scoliosis	β_0	-1.11	(.09)
	Age	-.57	(.34)
	Male gender	-.02	(.11)
Other neoplasms	β_0	-.95	(.23)
	Age	-4.07	(2.11)
	Male gender	-.06	(.21)
	Lisch nodules	-.55	(.25)
	Optic glioma	.32	(.31)

Figure 2: Adjusted intrafamilial latent correlation coefficients and their 95% confidence intervals for each of the 12 features among all 913 relatives with NF1 from the 373 families studied. In these estimates, all relatives are treated the same regardless of relationship.



Lisch nodules, head circumference, subcutaneous neurofibromas, cutaneous neurofibromas, stature, café-au-lait macules, and intertriginous freckling. Other correlations, such as for optic glioma and scoliosis, are positive, but not statistically different from zero however, the numbers are small and the confidence intervals are wide.

Using these methods, we repeated this analysis in four other ways: We examined the associations between features in all 1st and 2nd degree relatives, sibling pairs, all parent-child pairs, and father-child and mother-child pairs separately. Please see the Appendix, submitted manuscript C, Szudek et al, for detailed results. In summary, we found significantly greater correlations among 1st and 2nd degree relatives for Lisch nodules (p=.0001) and café au lait macules (p=.0004). Comparing sib-sib associations to those of parent-child, we found significantly greater correlations between sibs than between parents for subcutaneous neurofibromas (p=.04), café-au-lait macules (p=.001), intertriginous freckling (p=.03) and plexiform neurofibromas (p=.02), but not for Lisch nodules, head circumference, cutaneous neurofibromas, or stature. Comparing mother-child associations to father-child associations, we found that correlations between fathers and their children were significantly greater than mothers-child pairs for Lisch nodules (p=.001), subcutaneous neurofibromas (p=.0001) and cutaneous neurofibromas (p=.02).

We were able to postulate a number of pathogenic relationships from these findings. First, the significantly higher correlations among 1st than 2nd degree relatives for Lisch nodules is consistent with the effects of modifying genes and/or environmental factors. The sib-pair and parent-child associations were similar for Lisch nodules thus suggesting that modifying genes, rather than environmental factors are likely involved in the pathogenesis. We interpret the findings relating to café-au-lait macules in a similar way. The associations between these two features (from task 1) and the fact that both involve melanocytes, derived from neural crest tissue, may suggest that they share steps in pathogenesis. A parent of origin effect may explain the findings of a stronger association between fathers and children than mother and children for Lisch nodules, subcutaneous neurofibromas and cutaneous neurofibromas. Similar parent-child aggregation patterns have previously been reported for body mass index [Friedlander et al 1988] and blood pressure [Hurwich et al 1982] in the general population. We have no direct evidence of a genetic mechanism for these findings from our research in the current project. Features such as sub-cutaneous neurofibromas had significantly higher correlations between affected sibs than between affected parents and children. This pattern suggests an influence of the normal *NF1* allele on the phenotype observed.

The above analyses support the importance of genetic factors in determining the phenotypic expression of NF1 and also suggest that the nature of these factors differs for different features of NF1.

Head circumference and stature were variables with similar correlations for all relationships studied. This suggests that the mutant *NF1* allele itself is important in determining these correlations. Head circumference and stature have a strong and extensively researched genetic component in the general population. Thus, an important question for the present study was: Do all people with NF1 have larger heads and shorter stature than the general unaffected population, or is this effect due only to

certain mutant *NF1* alleles, in which case we would expect to see a bimodal distribution of head circumference and stature in people with NF1. This, and the apparent importance of *NF1* in growth regulation, lead us to an extensive study of growth patterns of people with NF1. The study [Szudek et al 2000b and 2000c] (both in the Appendix) included formal tests of modality and showed that the growth of all NF1 patients are affected, rather than particular sub-groups, and that the magnitude of the effect depends at least in part, on the mutant *NF1* allele.

Technical objectives 3 and 4: Derive age-specific risks.

Task 9: Calculate age-specific relative risks for true associations identified in individuals in Task 3.

Frequencies of the significant features of NF1 were calculated and presented in detail in Friedman and Birch, 1997a (Please see the Appendix). We also published many of these graphs (e.g for Café au Lait macules, Lisch nodules, axillary freckling, cutaneous/subcutaneous neurofibromas, optic glioma, and bony lesions) in DeBella et al, 2000a, a copy of which is included in this Appendix. The frequencies of UBOs have been published in DeBella et al 2000b, also included in the Appendix.

The associations between most of these features are insufficient to be useful clinically. The notable exception is for the association of optic gliomas with other gliomas of the central nervous system. We have demonstrated that in children under the age of 10 years at the time of their first imaging study, the association between optic glioma and other CNS tumours was highly significant (OR=24.5, 95%CI=3.2-1090). (Please see Friedman and Birch, 1997b, included in the Appendix).
All other associations have been discussed above and detailed in Szudek et al, 2000a, and submitted manuscript, "Unidentified Bright Objects on MRI Associated with Diagnostic Features in NF1").

Task 10: Calculate age-specific relative risks for familial associations.

We have not identified any features where the relative risk for familial associations is clinically useful. However, these familial associations are discussed in detail in submitted manuscript C.

Task 11: Complete data analyses and prepare scientific reports.

We have finished preparing all papers from the phenotype analysis. A number have been published, some have been accepted for publication or have been submitted. These are all included in the appendix and detailed in the Reportable Outcomes section, below.

The data analysis for the genotype-phenotype analysis has not yet been completed.

Technical objective 6: Identify allele-phenotype correlations between "familial phenotype" and allele type.

Task 1a: Set up techniques for strategic screening process for identification of constitutional mutations of NF1.

This task was completed during the first year of the project. (Please see year one Annual Report; Szudek et al., 1999a for details.)

Task 7 (Vancouver): Identify 40 patients for mutation identification from familial phenotypes identified in task 2. Contact contributing centres and obtain blood samples.

and

Task 2a (Salt Lake City): Identify mutations in these 40 NF1 patients.

Five phenotypes that were found to be familial were chosen for molecular analysis. These five phenotypes were described in detail in the year 2 report. During years 3 and 4, we obtained DNA or blood samples for mutation analysis from NF1 patients with the five phenotypes of interest. The number of samples obtained from patients in each are shown in the following table:

NF1 Phenotype	Number of Patients
Large deletion	11
Malignant peripheral nerve sheath tumours	6
NF1 vasculopathy	5
Optic glioma and other central nervous system gliomas	3
Late-diagnosed NF1, an initially mild phenotype	8

Molecular testing on these cases in Dr. David Viskochil's laboratory at the University of Utah is still in progress. These samples are being tested blindly by means of the multistage mutation identification process developed for this project.

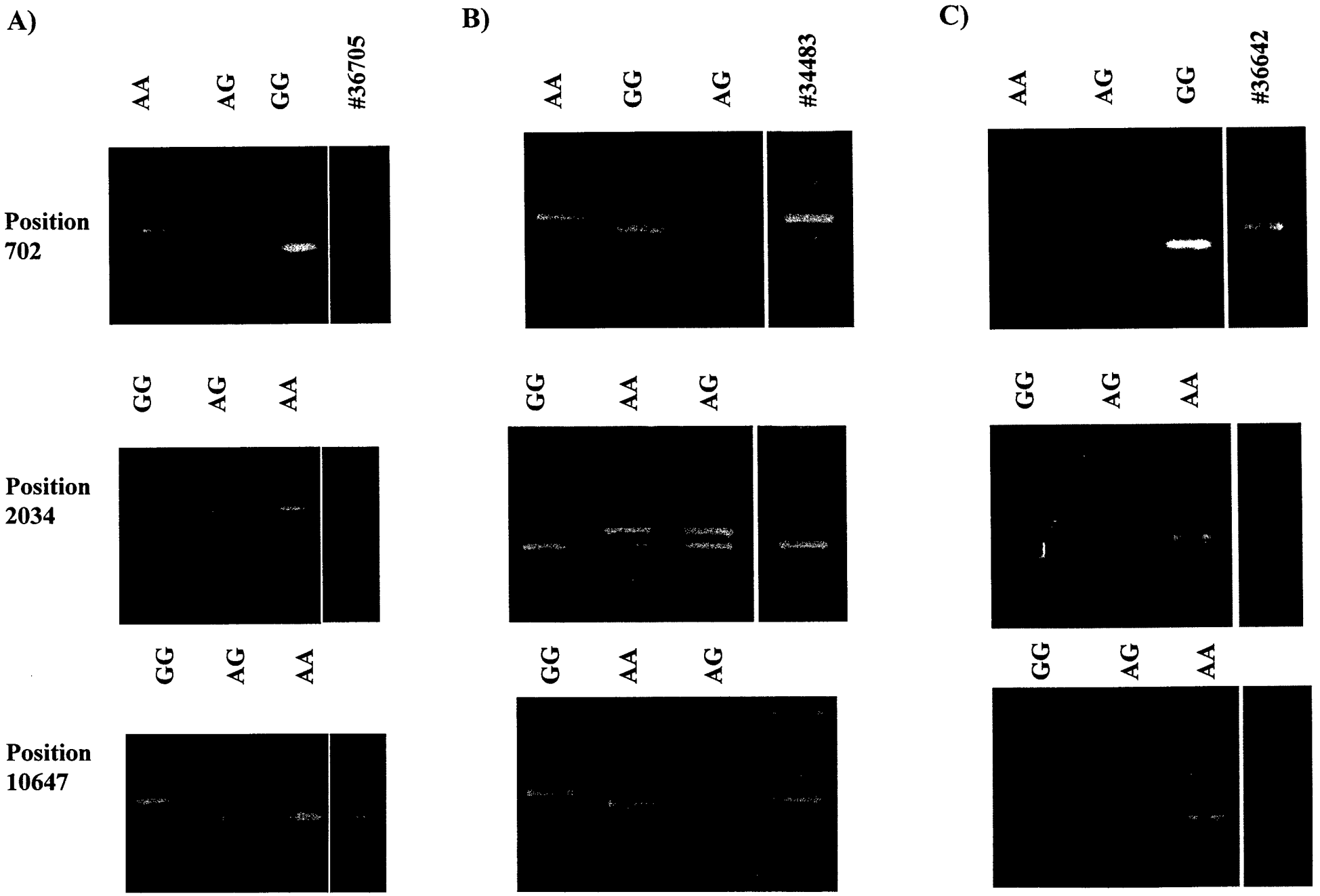
Of the 11 cases with a large deletion phenotype, 2 of 4 tested by FISH are deleted and two are not. PCR of 3 polymorphic intragenic markers has been completed on 6 of the 7 remaining cases, and only one was found to be heterozygous for one or more of these markers, ruling out a large deletion. Several of the PCR results are shown on figures 3-5. Population frequencies of heterozygosity for these polymorphisms range from 0.30 to 0.34 in whites, so it is likely that most of individuals who do not show heterozygosity for any of these markers are deleted. Given that large deletions are only responsible for about 5% of all NF1 cases, the finding of large deletions in at least 2/4 cases and perhaps as many as 7/10 cases selected on the basis of the phenotype alone is consistent with the existence of an allele-phenotype correlation.

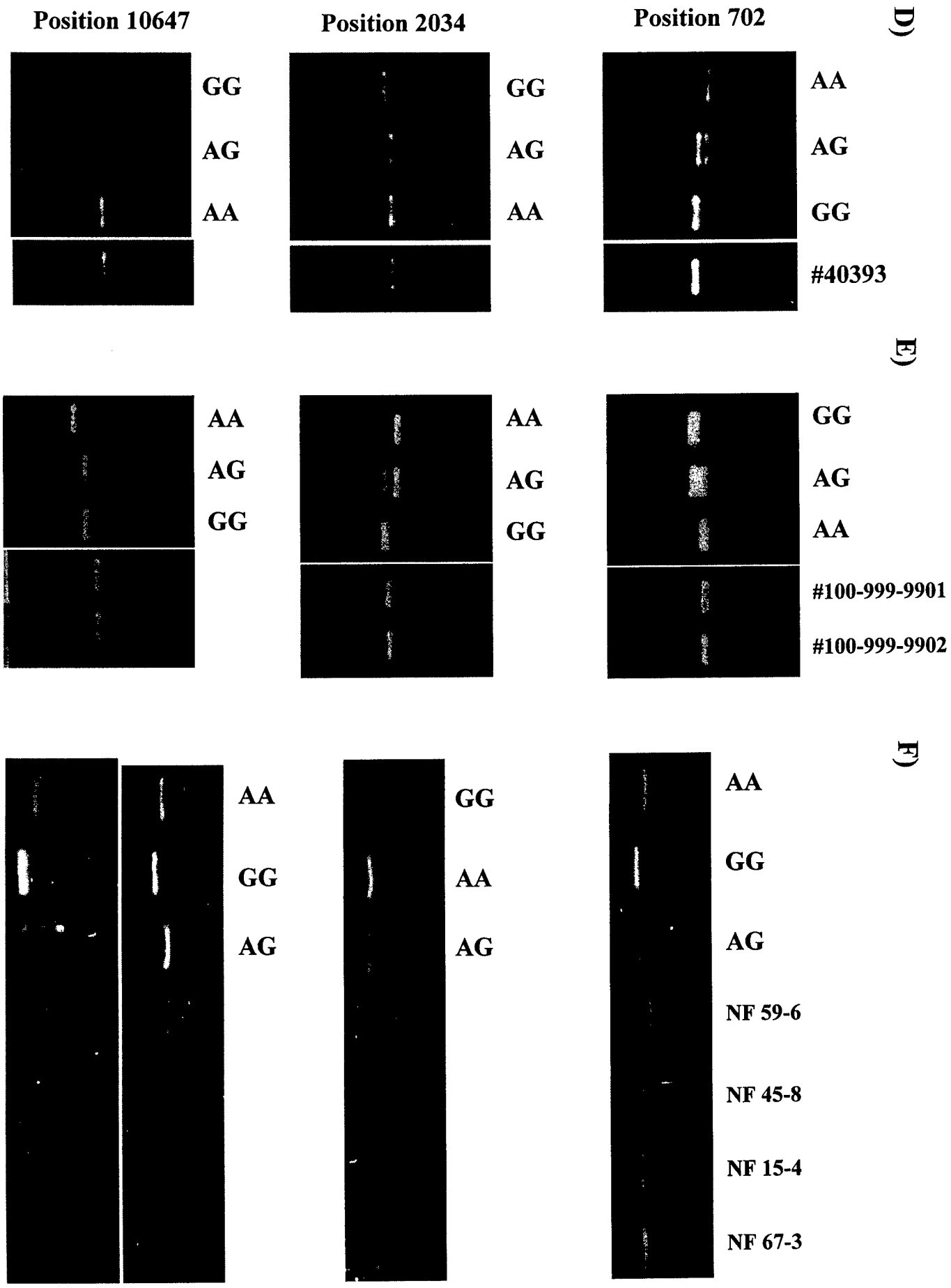
PCR products are being run and batched for sequencing on the specimens from patients with all of these phenotypes. Figure 4 shows examples for three of the PCR products from four of the samples. The remainder of the mutation testing, including full cDNA sequencing, will be completed on all of these specimens without further cost to the project.

Task 6: Review all cases with "familial phenotypes" in the NF1 Genetic Analysis Consortium Database and the published literature, looking for common mutations or mutation types.

Neither the NF1 Genetic Analysis Consortium Database nor the published literature provides detailed clinical descriptions on most patients on whom germline *NF1* mutations have been identified. We have recently received funding from the National Neurofibromatosis Foundation to develop state-of-the-art locus-specific databases for constitutional mutations of the *NF1* and *NF2* loci. This database will conform to current internationa' standards for locus-specific mutation databases and will include all published germline NF1 mutations as well as unpublished mutations that are made available by participating labs. In addition, we shall request standard minimal phenotypic data on all cases/families from the reporting labs. The experience we and others have gained through unsuccessful attempts to use the NF1 Genetic Analysis Consortium Database and existing published literature for genotype-phenotype correlations should help us to develop a resource that will facilitate such comparisons in the future.

Figures 3A-3H: MS-PCR results. AA, GG and AG controls are labelled accordingly. The top gels are results for position 702, the middle gels are for position 2034 and the bottom gels are results for position 10647. Experiments were carried out at least two times for each sample.


A)
AA
AG
GG
#36705
Position 702
GG
AG
AA
Position 2034
GG
AG
AA
Position 10647
B)
AA
GG
AG
#34483
GG
AA
AG
GG
AA
AG
C)
AA
AG
GG
#36642
GG
AG
AA
GG
AG
AA


D)
Position 10647
Position 2034
Position 702
GG
AG
AA
GG
AG
AA
AA
AG
GG
#40393
E)
AA
AG
GG
AA
AG
GG
GG
AG
AA
#100-999-9901
#100-999-9902
F)
AA
GG
AG
GG
AA
AG
AA
GG
AG
NF 59-6
NF 45-8
NF 15-4
NF 67-3
repeat experiment
First experiment

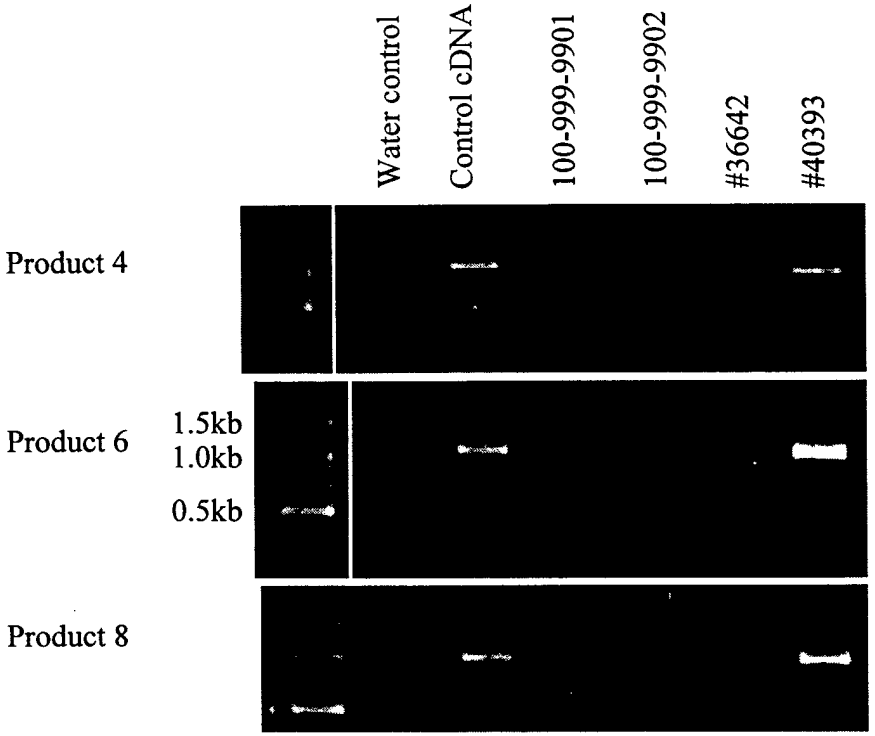
G)

Position 702
AA
GG
AG
NF 16-03
NF 70-03
NF 57-03
NF 42-03
NF 21-4
Position 2034
Positive controls were very faint
Position 10647
AA
GG
AG
Last sample did not work
Repeat with the last sample

H)

AA
AG
GG
AA
AG
GG
AA
AG
GG
NF 60-03
NF 135-01
Controls did not work, not certain of data

Figure 4. NF-1 PCR products 4, 6 & 8 for samples 100-99-9901, 100-999-99902, #36642 & #40393



NF-1PCR products 2, 3, 5, 7, 9 & 11 for sample #40393

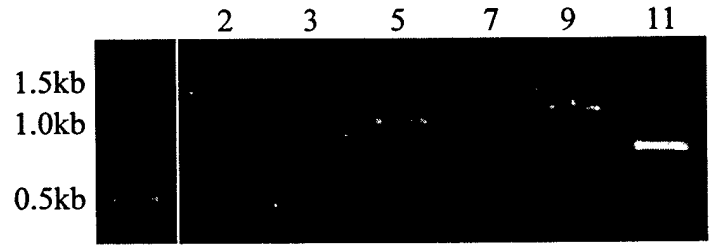
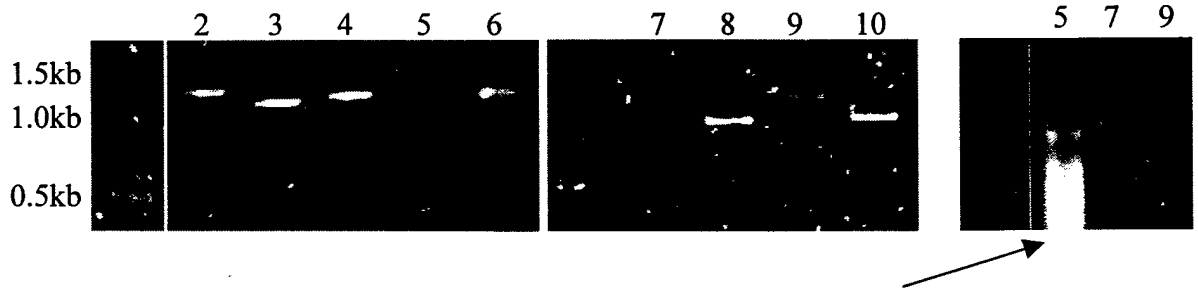


Figure 4. NF-1 PCR products 2-10 for sample #34483



Repeat for products that did not work the first time with the exception of product 9

Figure 5. NF-1 PCR products 2 to 11 for samples NF 59-6, NF 45-8, NF 15-4 and NF 67-3

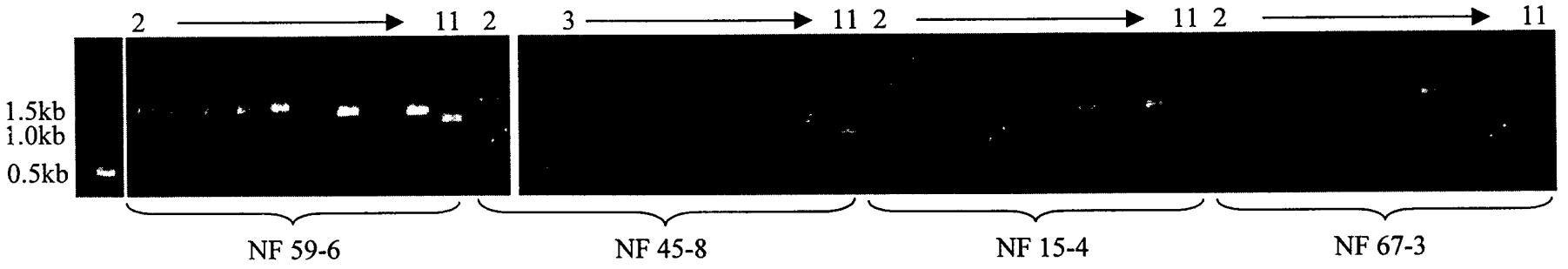
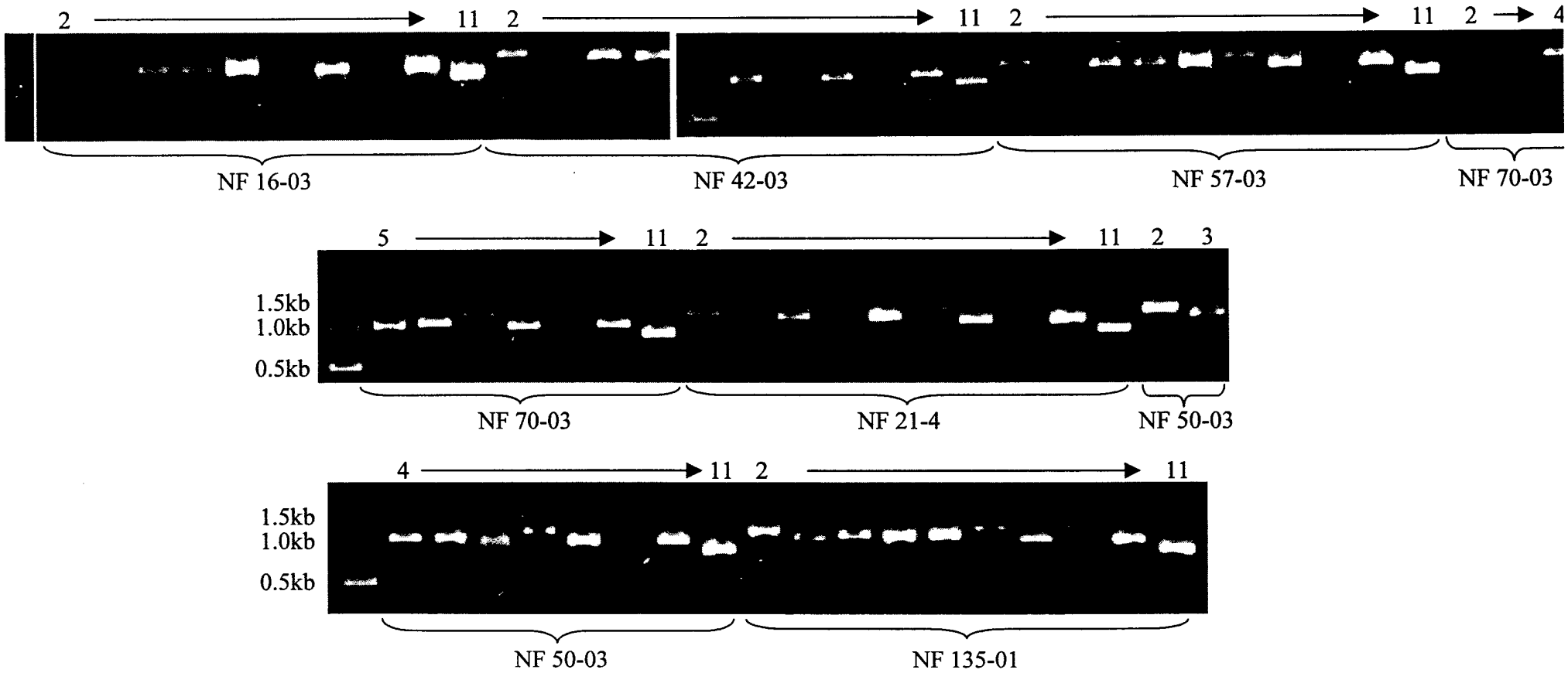


Figure 6. NF-1 PCR products 2 to 11 for samples NF 16-03, NF 42-03, NF 57-03, NF 70-03, NF 21-4, NF 50-03 and NF 135-01



KEY RESEARCH ACCOMPLISHMENTS

- Demonstration of associations among clinical features in NF1 patients using stratified Mantel-Haenszel analysis and logistic regression to control for the confounding effect of age.
- Demonstration of associations of NF1 clinical features among family members using logistic regression and multivariate probit models to control for the confounding effect of age.
- Identification of phenotypic groups and relationships between important features of NF1
- Identification of familial phenotypes as candidates for allele-phenotype correlations.
- Development and validation of exhaustive molecular screening process for pathogenic mutations of the *NF1* locus.
- Testing of NF1 patients with candidate familial phenotypes for allele-phenotype correlations is currently in progress.
- Proof of principle for analysis of *NF1* allele-phenotype associations - Deletion phenotype analysis

REPORTABLE OUTCOMES

1. Manuscripts, abstracts and presentations: (All abstract, reprints, and unpublished manuscripts are included in the appendix).

Papers published in peer-reviewed scientific journals (meeting presentations and abstract publications as noted*)

Baser ME, Birch PH, Evans GR, Friedman JM: **Association of superficial plexiform and paraspinal neurofibromas in neurofibromatosis 1.** *Neurology* 1999 Apr 22;52(7):1519-20

DeBella K, Poskitt K, Szudek J, Friedman JM. **Use of "unidentified bright objects" on MRI for diagnosis of neurofibromatosis 1 in children.** *Neurology* 54:1646-1651, 2000b.
*Oral presentation: American Society of Human Genetics, October 1999
*Published abstract: *Am J Hum Genet* 65(3) A36 Supplement, 1999

DeBella K, Szudek J, Friedman JM. **Use of the National Institutes of Health criteria for diagnosis of neurofibromatosis 1 in children**. *Pediatrics* 105:608-14, 2000a.
*Published abstract: *Am J Hum Genet* 63(4) A101 Supplement, 1998

Friedman JM, Birch P. **An association between optic glioma and other tumours of the central nervous system in neurofibromatosis type 1**. *Neuropediatrics* 28:131-132, 1997.

Friedman JM, Birch PH: **Type 1 Neurofibromatosis: A descriptive analysis of the disorder in 1,728 patients.** *Am J Med Genetics* 70:138-143, 1997

Friedman JM. **Epidemiology of NF1.** *Am J Med Genet* 89:1-6, 1999

Hamilton SJ, Friedman JM. **Insights into the pathogenesis of neurofibromatosis 1 vasculopathy**. *Clin Genet* 58:341-344, 2000

Hamilton SJ, Allard MF, Friedman JM. **Cardiac findings in an individual with neurofibromatosis 1 and sudden death**. *Am J Med Genet* 100:95-9, 2001.

Lin AE, Birch PH, Korf BR, Tenconi R, Niimura M, Poyhonen M, Armfield Uhas KA, Sigorini M, Virdis R, Romano C, Bonioli E, Wolkenstein P, Pivnick E, Lawrence M, Friedman JM. **Cardiovascular malformations and other cardiac abnormormalities in neurofibromatosis 1.** *Am J Med Genet* 95:108-117, 2000.
*Oral presentation: National Neurofibromatosis Foundation Annual Symposium, October 1999.

Rasmussen SA, Friedman JM. **NF1 gene and neurofibromatosis type 1.** *Am J Epidemiol* 151:33-40, 2000.

Rasmussen SA, Yang Q, Friedman JM: **Mortality in neurofibromatosis 1: An analysis using U.S. death certificates.** *Am J Hum Genet* 68:1110-1118, 2001
*Oral presentation: American Society of Human Genetics, October 1999
*Published abstract: *Am J Hum Genet* 65(3) A49 Supplement, 1999

Stevenson DA, Birch PH, Friedman JM, Viskochil DH, Balestrazzi P, Boni S, Buske A, Korf BR, Niimura M, Pivnick EK, Schorry EK, Short MP, Tenconi R, Tonsgard JH, Carey JC: **Descriptive analysis of tibial pseudarthrosis in patients with neurofibromatosis 1.** *Am J Med Genet* 1999 Jun 11;84(5):413-9
*Oral presentation: National Neurofibromatosis Foundation Annual Symposium, October 1998

Szudek J, Birch PH, Friedman JM, Participants of the NNFF International Database. **Growth in North American white children with neurofibromatosis 1.** *J Med Genet* 37:933-938, 2000b.
*Published abstract: *Am J Hum Genet* 63(4) A122 Supplement, 1998

Szudek J, Birch P, Riccardi VM, Evans DG, Friedman JM. **Associations of clinical features in neurofibromatosis 1.** *Genet Epidemiol* 19:429-439, 2000a.
*Published abstract: *Am J Hum Genet* 61 A115 Supplement, 1997

Szudek J, Birch P, Friedman JM. **Growth charts for young children with neurofibromatosis 1 (NF1)** . *Am J Med Genet* 92:224-228, 2000c.

Tzenova J, Joe H, Riccardi VM, Friedman JM. **The effect of parental age on the occurrence of Neurofibromatosis 1.**
*Published abstract: *Am J Hum Genet* 69(4) A1229 Supplement, 2001

Papers submitted, accepted for publication, or in-press (meeting presentations and abstract publications as noted*)

Kwok ESH, Sawatzky B, Birch P, Friedman JM, Tredwell SJ: **Vertebral scalloping in neurofibromatosis 1: A quantitative approach**. *Canad J Surg* (in press).
*Published abstract: *Am J Hum Genet* 67(4) A210 Supplement, 2000

Szudek J, Friedman JM. **Unidentified Bright Objects on MRI Associated with Diagnostic Features in Neurofibromatosis 1 (NF1).** Accepted for publication, *Pediatric Neurology.*

Szudek J, Joe H, Friedman JM: **Logistic Regressive Models of Neurofibromatosis 1 (NF1) Features.** Submitted for publication.
*Oral presentation: American Society of Human Genetics, October 1999
*Poster presentation: 8th European Neurofibromatosis Symposium, Ulm, Germany, September 1999
*Published abstract: *Am J Hum Genet* 65(3) A36 Supplement, 1999

Palmer V, Szudek J, Joe H, Riccardi VM, Friedman JM. **Analysis of Neurofibromatosis 1 (NF1) Lesions by body segment.** Submitted for publication.
*Published abstract: *Am J Hum Genet* 67(4) A132 Supplement, 2000

Szudek J, Joe H, Friedman JM. **Analysis of Intra-Familial Phenotypic Variation in Neurofibromatosis 1 (NF1).** Submitted for publication.
*Published abstract: *Am J Hum Genet* 67(4) A211 Supplement, 2000

Online Publications

Friedman, JM. Neurofibromatosis Type 1. In GeneClinics, HTTP://www.geneclinics.org/nf1.html

Rasmussen SA, Friedman JM. NF1 gene and neurofibromatosis type 1. In HuGENet, HTTP://www/cdc.gov/genetics/hugenet/NF1gene.htm (1999).

Additional Presentations

Invited Speaker Clinical Manifestations and Diagnostic Criteria of NF1. In Child Neurology Society, Neurobiology of Disease in Children Symposium. Oct. 2001.

Invited Speaker, Cardiovascular manifestations of neurofibromatosis. BC Neurofibromatosis Foundation, Burnaby, BC, February 2001

Invited Speaker, Clinical aspects of neurofibromatosis. Washington Chapter, National Neurofibromatosis Foundation, Seattle, Washington, April 2001

Invited Speaker, Workshop on Malignant Peripheral Nerve Sheath Tumours in NF1, London, UK, May 2001

2. Degrees obtained supported by this award

- Jacek Szudek, Ph.D. (full support)
- Kim DeBella, B.Sc. (partial support; now completing MD degree)
- Chana Palmer, B.Sc. (partial support; now completing Ph.D. degree)
- Jordana Tzenova, M.Sc. candidate (partial support)
- Sara Hamilton, M.Sc (partial support)

3. Informatics resources:

- The NNFF International Database was converted from a DOS-based Clipper database to MS-Access, and facilities were developed to permit data entry via the World Wide Web or through scannable paper forms. These systems have all been fully tested and implemented.

- The NF Institute Database was originally compiled on paper forms. Some of the information from each patient's first and most recent visits was recorded in an electronic database several years ago. We have recently transferred these data to MS-Access and have entered additional quantitative and family data from the paper forms. This new database has provided crucial information for our studies of familial aggregation of various traits in NF1 patients. The resource has also been used by other researchers for related studies.

4. Funding applied for based on work supported by this award

- One students, a US citizen, received a salary matching training grant from the Government of British Columbia, Department of Science and Technology.
- Another student, also a US citizen, received a Summer research award from the British Columbia Children's Hospital Foundation
- One student received two University Graduate Fellowships as a result of publications related to this grant.

5. Employment and training based on experience/training supported by this award

Three undergraduate students supported by this award have gone on to graduate degrees.
One graduate student is now taking a further graduate degree.

6. Personnel receiving pay from the research effort

J.M. Friedman, Lab Director
Patricia Birch, Research Scientist
Daphne Savoy, Research Technician
Carmen Larsen, Research Technician
Tiffany Britton, Research Technician
Jacek Szudek, student
Kim DeBella, student
Chana Palmer, student
Jordana Tzenova, student
YinShan Xao, student
Jeff Low, summer student

CONCLUSIONS

During the project's first year, we used association studies to identify subgroups of NF1 patients in which at least some of the phenotypic variability appears to result from genetic factors, and we set up molecular techniques to identify *NF1* gene mutations.

During the second year, statistical methods, including logistic regression, generalized estimating equations and multivariate normal models were used to analyse associations while controlling for the confounding affect of age. In this year, proof of principal was begun for allele-phenotype associations using the "deletion phenotype" as a model.

In the third year, we began to collect blood samples and identify patients who would be appropriate to study for allele-phenotype correlations. The strategic screening process was modified to include processing of RNA to produce cDNA. We also used more sophisticated statistical methods, including multivariate probit modelling, in order to investigate the sources of phenotypic variability for certain familial features of NF1. We were able to differentiate between features which appeared to be influenced by the *NF1* allele from those influenced by the normal *NF1* allele, and by modifying genes unlinked to *NF1*.

In the fourth and final year, we shall finish the allele-phenotype correlational studies and will continue to examine other possible effects such as the epigenetic influences of imprinting.

These studies are providing considerable insight into the causes of clinical variability of NF1. An understanding of the reasons for this clinical variability is a necessary prerequisite to understanding the genetics of NF1 and is central to genetic counselling as well as to development of new approaches to therapy.

APPENDIX

confirmation will permit the establishment of patient cohorts for observational studies as well as therapeutic trials.

David W. Desmond, PhD, Joan T. Moroney, MD, MRCPI, *New York, NY*

References

1. Desmond DW, Moroney JT, Lynch T, et al. CADASIL in a North American family: clinical, pathologic, and radiologic findings. Neurology 1998; 51:844–849.
2. Chabriat H, Vahedi K, Iba-Zizen MT, et al. Clinical spectrum of CADASIL: a study of seven families. Lancet 1995;346:934–939.
3. Desmond DW, Moroney JT, Lynch T, Chan S, Chin SS, Mohr JP. Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL): a meta-analysis of previously published cases. Neurology 1998;50(suppl 4):A441–A442. Abstract.
4. Dichgans M, Mayer M, Uttner I, et al. The phenotypic spectrum of CADASIL: clinical findings in 102 cases. Ann Neurol 1998;44:731–739.
5. Chabriat H, Levy C, Taillia H, et al. Patterns of MRI lesions in CADASIL. Neurology 1998;51:452–457.
6. Ruchoux MM, Maurage CA. CADASIL: cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. J Neuropathol Exp Neurol 1997;56:947–964.
7. Vérin M, Rolland Y, Landgraf F, et al. New phenotype of the cerebral autosomal dominant arteriopathy mapped to chromosome 19: migraine as the prominent clinical feature. J Neurol Neurosurg Psychiatry 1995; 59:579–585.
8. Bergmann M, Ebke M, Yuan Y, Brück W, Mugler M, Schwendemann G. Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL): a morphological study of a German family. Acta Neuropathol 1996;92:341–350.
9. Nishio T, Arima K, Eto K, Ogawa M, Sunohara N. [Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy—report of an autopsied Japanese case]. Rinsho Shinkeigaku 1997; 37:910–916.

Infarction in the territory of the anterior cerebral artery

To the Editor: Klatka et al. claim that their patient exhibits "severe neglect of the left side."[1] They may be overlooking an important point about the role of mesial frontal regions in spatial attention. What they call a line bisection task (their figure 1A) actually reveals omission of marks from the left side of *an array* of lines, a performance supporting their contention of left-sided or contralesional neglect (CN). Close inspection of *individual lines,* however, shows consistent leftward bisection errors, a finding consistent with neglect of the right side of the line or ipsilateral neglect (IN).[2]

We have observed patients with similar behavior after right frontal brain injury.[3] Namely, they misbisected lines to the left of center but, paradoxically, neglected the left side of stimuli on other tasks (e.g., target cancellation). Following the theoretical framework of Denny-Brown and Chambers,[4] leftward bisection error might result from release of "approach" behavior, whereby impulsive response to or "visual grasp" of contralesional stimuli impels midpoint judgments to the left of true midpoint. However, because the prefrontal cortex also contributes to a network responsible for directed attention,[5] right frontal lesions may also cause inattention for left-sided stimuli, negating the influence of disinhibited approach behavior.

Hence, we propose that task performance after right frontal lesions may reflect a dynamic antagonism between visual grasp toward the left and attentional bias toward the right. If demands for processing of local stimulus features increase (e.g., array cancellation), the resulting bias favors right space and causes CN. Alternatively, spatial stimuli that emphasize processing of global features (e.g., line bisection) should result in bias to the left (IN). The behavior depicted in their figure 1A[1] supports our proposal, demonstrating "severe neglect of the left side" of the array of items while simultaneously showing bias toward the left half of individual lines.

John C. Adair, MD, *Albuquerque, NM;* Kenneth M. Heilman, MD, *Gainesville, FL;* Duk L. Na, MD, *Seoul, Korea*

Reply from the Authors: We thank Drs. Adair, Heilman, and Na for their comments regarding IN. The focus of our article was, in part, to describe prominent contralesional sensory neglect in a patient with a nondominant anterior cerebral infarction. The respondents correctly point out that our patient is also displaying IN, which has been associated with frontal lesions and has been attributed to disinhibition of right-sided attentional systems, driving the response to the contralesional side.[2] Other authors have noted variation in the pattern of line bisection with stimulus size, with long lines bisected to the right of midpoint and shorter lines bisected to the left of midpoint. These patients had nondominant lesions in a variety of locations, including but not limited to the frontal lobe.[6] The reason for this observation was unclear, but possible mechanisms included "perceptual completion," or completion of the image to the left of the fixation point. Another possible explanation for IN involves impaired exploration in the ipsilateral hemispace after right hemisphere injury (but not left hemisphere injury), supporting the concept of right hemispheric dominance for spatial distribution of attention.[7] Our finding of both CN and IN in a single task supports this theory, but also suggests that there may be multiple mechanisms governing spatial distribution of attention.

Lisa A. Klatka, DO, *Washington, DC*

References

1. Klatka LA, Depper MH, Marini AM. Infarction in the territory of the anterior cerebral artery. Neurology 1998;51:620–622.
2. Kwon SE, Heilman KM. Ipsilateral neglect in a patient following a unilateral frontal lesion. Neurology 1991;41:2001–2004.
3. Kim M, Na DL, Kim GM, Adair JC, Lee KH, Heilman KM. Ipsilesional neglect: behavioral and anatomic features. J Neurol Neurosurg Psychiatry 1999 (in press).
4. Denny-Brown D, Chambers RA. The parietal lobe and behavior. Assoc Res Nerv Ment Dis Proc 1958;36:35–117.
5. Watson RT, Valenstein E, Heilman KM. Thalamic neglect. Possible role of the medial thalamus and nucleus reticularis in behavior. Arch Neurol 1981;38:501–506.
6. Tegner R, Levander M. The influence of stimulus properties on visual neglect. J Neurol Neurosurg Psychiatry 1991;54:882–887.
7. Weintraub S, Mesulam MM. Right cerebral dominance in spatial attention. Further evidence based on ipsilateral neglect. Arch Neurol 1987;44: 621–625.

Association of superficial plexiform and paraspinal neurofibromas in neurofibromatosis 1 (NF1)

To the Editor: Tonsgard et al.[1] performed CT imaging in 91 adult (≥16 years) NF1 patients; paraspinal neurofibromas occurred in 18.7% of patients in the chest region and in 25.3% of patients in the abdominal or pelvic region. The majority of patients were asymptomatic.

We wondered whether the presence of plexiform neurofibromas that are apparent on the surface of the body could be used to identify NF1 patients who are at high risk of having paraspinal neurofibromas as well. We therefore examined the association between NF1 superficial plexiform and paraspinal neurofibromas in both the National Neurofibromatosis Foundation International Database (NNFFID; 2,638 patients)[2,3] and an English series (288 patients).

Superficial plexiform neurofibromas occurred in 23.2% of NNFFID patients and 24.0% of English patients. Paraspinal neurofibromas occurred in 1.7% of NNFFID patients and 4.2% of English patients. The low frequency of paraspinal tumors is probably due to failure to identify most asymptomatic tumors, compounded by a pediatric ascertainment bias in the NNFFID (61.8% of patients were <16 years old).

Using the NNFFID data, we age- and sex-matched NF1 patients with plexiform neurofibromas 1:2 with NF1 patients who lacked plexiform neurofibromas. In all age groups, 3.3% of patients with plexiform neurofibromas had paraspinal neurofibromas, compared to 1.3% of patients without plexiform neurofibromas (odds ratio [OR] = 2.6; 95% confidence interval [CI] = 1.4 to 4.9). In patients <16 years old, 3.5% of patients with plexiform neurofibromas had paraspinal neurofibromas, compared to 0.7% of patients without plexiform neurofibromas (OR = 5.4; 95% CI = 2.0 to 15.2).

In the English series, 8.7% of patients with plexiform neurofibromas had paraspinal neurofibromas, compared to 2.7% of patients without plexiform neurofibromas (OR = 3.4; 95% CI = 0.9 to 13.1). There were too few young patients to conduct an age-stratified analysis. In both the NNFFID and the English series, no

other abnormalities were significantly overrepresented in patients with plexiform neurofibromas.

Although the biological basis for an association between surface and paraspinal neurofibromas is unknown, there are several possibilities (which are neither mutually exclusive nor the only ones). Easton et al.[4] modeled NF1 traits to determine if the variable expression of NF1 had an inherited component. They concluded that NF1 trait-specific phenotypes (including plexiform neurofibromas) were largely determined by genotypes at modifying loci, although a precise model could not be specified. Some patients may have a form of NF1 that highly predisposes to the development of plexiform neurofibromas involving both superficial structures and spinal nerves and ganglia. These patients might have a higher-than-expected paraspinal and superficial plexiform tumor count based on age, analagous to *NF1* deletions and a large number of neurofibromas based on age.[5] In this case, the lesions would not necessarily occur in the same area of the spine. Paraspinal neurofibromas might secrete a factor that causes overgrowth of susceptible tissues in a paracrine fashion, so that a plexiform tumor that lies adjacent to a spinal nerve root or ganglion might promote the proliferation of the cells to become a paraspinal tumor. This mechanism would produce surface and paraspinal plexiform neurofibromas that are spacially contiguous. Our data on tumor location are not detailed enough to test the last two hypotheses, but future research may do so.

Although we observed an association between surface plexiform and paraspinal neurofibromas, the sensitivity and specificity were not high enough for the association to be used clinically to identify individual patients at high risk of paraspinal neurofibromas. Further research is necessary to define the natural history of paraspinal neurofibromas in NF1 patients and to determine the most effective means of identifying patients who are likely to develop serious complications from these lesions.

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Reply from the Authors: I agree with Dr. Baser et al. that there is an association of superficial plexiform neurofibromas with deep internal lesions in some patients. That association has not been adequately delineated. Although our experience indicates that the tissue diagnosis can be predicted in the vast majority of internal lesions, superficial lesions are more problematic. Isolated dermal or cutaneous neurofibromas, diffuse neurofibromas, and plexiform lesions may be difficult to distinguish in the absence of a biopsy. Because we do not have biopsy information on the majority of our patients with superficial lesions, I indicated in our article the number of patients with superficial lesions, without attempting to analyze that group further or examine their association with deep plexiform lesions.

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References

1. Tonsgard JH, Kwak SM, Short MP, Dachman AH. CT imaging in adults with neurofibromatosis-1. Neurology 1998;50:1755–1760.
2. Friedman JM, Birch PH, Greene C, NNFF International Database participants. National Neurofibromatosis Foundation International Database. Am J Med Genet 1993;45:88–91.
3. Friedman JM, Birch PH. Type 1 neurofibromatosis: a descriptive analysis of the disorder in 1,728 patients. Am J Med Genet 1997;70:138–143.
4. Easton DF, Ponder MA. Huson SM, Ponder BAJ. An analysis of variation in expression of neurofibromatosis (NF) type 1 (NF1): evidence for modifying genes. Am J Hum Genet 1993;53:305–313.
5. Kayes LM, Burke W, Riccardi VM, et al. Deletions spanning the neurofibromatosis 1 gene: identification and phenotype of five patients. Am J Hum Genet 1994;54:424–436.

Antibodies from ALS patients inhibit dopamine release mediated by L-type calcium channels

To the Editor: Offen et al.[1] claim to have produced evidence that "... confirms the presence of antibodies against the L-type calcium channel in the majority of ... ALS patients, supporting their role in the pathogenesis of ALS."[1] Controversy surrounds the question of anti-voltage-gated calcium channel (VGCC) antibodies in ALS and the data presented do not justify these conclusions.

VGCCs are a family of genetically heterogeneous proteins that exhibit regional and functional specialization. The α_{1S} subunit, for example, forms an L-type VGCC that is important in mediating excitation-contracting coupling in skeletal muscle, and the α_{1A} subunit forms a P/Q-type VGCC that is the primary mediator of excitation-secretion coupling in human spinal motor neurons.

Appel et al. reported that ALS immunoglobulin (Ig) preparations both *increased* miniature end plate potential (MEPP) frequency at the mouse neuromuscular junction (NMJ) and *decreased* calcium currents,[2] a discrepancy for which no adequate explanation was offered. The presence of antibodies to rabbit skeletal muscle L-type VGCCs[3] that specifically bind to the α_1 subunit of these L-type VGCCs[4] were subsequently described. ALS Ig preparations have since been shown to increase P/Q-type currents in cerebellar Purkinje cells[5] and to be cytotoxic to a rat motor neuron/mouse neuroblastoma cell line, an effect that was overcome by blockade of N- or P-type VGCC currents.[6] Anti-P/Q- and anti-N-type VGCC antibodies have been detected in a minority of ALS patients,[7] but other investigators have been unable to confirm these results.[8,9] Moreover, the initial report of antibodies directed against the α_1-subunit of the VGCC was based on the ability of ALS Ig preparations to inhibit the binding of a monoclonal antibody to the α_1-subunit[4] and it has subsequently been suggested that the contamination of serum preparations with the serum protease, plasmin, may produce the same effect.[10]

It is against this controversial background that the effects of ALS Ig preparations on ^{3}H-dopamine release from PC12 cells have been examined.[1] Although it is true that the bulk of exocytosis from these cells is mediated by L-type VGCCs, an inhibitory effect on depolarization evoked dopamine release does not necessarily implicate the VGCC. Two forms of neurotransmitter release are recognized. Basal release from the resting nerve terminal accounts for the physiologically measured spontaneous MEPPs and action potential depolarization of the nerve terminal leads to calcium influx via VGCCs and the activation of the presynaptic exocytotic cascade, which involves many proteins including synaptobrevin, syntaxin, SNAP-25, and synaptotagmin. Moreover, both of these processes are influenced by the resting membrane potential, which may be disturbed by alterations in the function of voltage gated potassium channels and the Na^+-K^+ ATPase. A physiologic effect on neurotransmitter release may, therefore, reflect interference with the function of any of these processes. Offen et al.[1] have not addressed these possibilities.

The inhibitory effect on VGCC-dependent evoked neurotransmitter release described by Offen et al.[1] does not provide an explanation for the observed increase in MEPP frequency,[2] the increased P-type currents,[5] or the cytotoxic effect on the motor neuron/neuroblastoma cells.[6] Moreover, an inhibitory effect on α_{1C} L-type VGCCs would not provide an adequate explanation for the death of motor neurons observed in ALS.

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Reply from the Authors: In our study,[1] we applied protein-A purified ALS IgG to PC12 cells and showed a reduction in depolarization evoked [^{3}H]dopamine release and no change in basal release.

The reduction in depolarization evoked release by externally applied IgG fraction must be the result of an interaction with extracellular epitopes, excluding any direct interaction with syntaxin, SNAP-25, or synaptotagmin or other intracellular proteins. Whereas the VGCC are crucial for initiating evoked release, neither the voltage sensitive K^+-channel nor the Na^+/K^+ ATPase play any role in the onset of the release process.

VGCCs are multi-transmembrane proteins, recently shown to be functionally associated with the exocytotic proteins, syntaxin, SNAP-25, and synaptotagmin in an excitosome complex.[11-14] We proposed that extracellular domains of the Lc-type channel, the L-type C-class channel present in PC12 cells, interact with ALS IgG to modify the release process,[1] most likely interfering with VGCC coupling to the release machinery within the excitosome.[13,14]

Unlike previous binding experiments,[4] the presence of anti-VGCCs in ALS IgG fraction was demonstrated in a functional assay.[1] Furthermore, by using protein-A purified preparation,[1] we excluded the possible interference of IgG impurities as previously suggested.[10]

protein genotype and sporadic Creutzfeldt-Jakob disease. Lancet 1999;353:1673–1674.
35. Will RG, Ironside JW, Zeidler M, et al. A new variant of Creutzfeldt-Jakob disease in the UK. Lancet 1996;347:921–925.
36. Chazot G, Broussolle E, Lapras CI, Blätter T, Aguzzi A, Kopp N. New variant of Creutzfeldt-Jakob disease in a 26-year-old French man. Lancet 1996;347:1181.
37. Birchard K. Variant Creutzfeldt-Jakob disease found in Ireland. Lancet 1999;353:2221.
38. Ironside J. Prion diseases in man. J Pathol 1998;186:227–234.

Use of "unidentified bright objects" on MRI for diagnosis of neurofibromatosis 1 in children

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Article abstract—*Background:* "Unidentified bright objects" (UBOs) have been observed as areas of increased T2-weighted signal intensity on MRI in 43% to 93% of children with neurofibromatosis 1 (NF1). Because of this high frequency and the fact that the NIH diagnostic criteria often do not permit diagnosis of NF1 in early childhood, UBOs have been proposed as an additional diagnostic criterion. *Methods:* The authors examined the sensitivity and specificity of UBOs for NF1 in 19 affected children and 19 age-matched controls. Eleven of the control patients had CNS pathology that might be classified as UBOs on MRI scan. The authors measured the agreement in recognition of UBOs between two pediatric neuroradiologists who independently examined the MRI studies of these patients. They also assessed the effect of adding UBOs to the NIH diagnostic criteria on ability to diagnose NF1 in young patients. *Results:* Sensitivity and specificity of UBOs for NF1 averaged 97% and 79%, respectively. Agreement between the two radiologists was 84% overall, but varied greatly from one brain region to another. Adding UBOs as a diagnostic criterion permitted the diagnosis of 30% of young patients with *NF1* mutations who do not meet the existing NIH diagnostic criteria for NF1. The value of including UBOs is less in older patients because a larger proportion of them meet the existing diagnostic criteria. *Conclusions:* UBOs are difficult to identify with certainty and occur in children with suspected CNS disorders who do not have NF1, but they tend to occur in different brain regions. UBOs may be more useful for diagnosis of NF1 in young children if they can be defined precisely and if only the cerebellum, brainstem, and basal ganglia are considered. **Key words:** Neurofibromatosis 1—Unidentified bright objects—Diagnosis—MRI.

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Neurofibromatosis 1 (NF1) is diagnosed by using a set of clinical criteria that was developed by a NIH Consensus Conference in 1988[1] and recently reaffirmed by another group of experts.[2] These criteria are thought to be very reliable in distinguishing adults with and without NF1 on the basis of routine clinical and ophthalmologic examinations.[3] However, most of the clinical features on which the diagnosis is based are uncommon in infants with NF1 and increase in frequency with age.[4] As a consequence, >40% of 2-year-old children with sporadic NF1 cannot be diagnosed by using the NIH criteria.[4]

The NIH diagnostic criteria were developed before MRI was commonly used as a screening technique in patients with NF1.[2] Many features of NF1, including optic gliomas, orbital neurofibromas, other gliomas, and "unidentified bright objects" (UBOs) are apparent on cranial MRI examination.[5] UBOs are areas of increased T2-weighted signal intensity that cannot be visualized on T1-weighted imaging.[6] They show no mass effect or contrast enhancement.[7] Pathologically, they correspond to areas of vacuolar or spongiotic change in the brain substance.[8] UBOs are found in 43% to 93% of pediatric NF1 patients.[9,10] UBOs evolve over time and are more common in children than in adults with NF1.[11] No consistent relation has been found between the learning disabilities that frequently occur in NF1 patients and the presence of UBOs, although a correlation between the location of these lesions and IQ score has been suggested.[12]

Because they are so common in pediatric patients with NF1, UBOs have been proposed as an additional diagnostic criterion in young patients.[13] Ear-

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lier diagnosis of NF1 would be beneficial to both affected children and their families. Genetic counseling could be offered to parents and other relatives earlier, and interventions for learning or developmental problems could be initiated sooner.[14] Conversely, MRI studies are expensive and require anesthesia in small children. The use of UBOs to diagnose NF1 also has been criticized because the specificity of UBOs to NF1 patients is unknown.[2] Although UBOs are thought to be uncommon in children who do not have NF1,[13] the frequency in normal children also is unknown. Lesions that resemble UBOs have occasionally been reported in adults who do not have NF1.[15,16]

We examined the sensitivity and specificity of UBOs for NF1 and measured the agreement between two radiologists who independently examined the MRI studies on a series of NF1 patients and age-matched controls. We also assessed the effect of adding UBOs to the NIH diagnostic criteria on our ability to diagnose NF1 in young patients.

Methods. Two groups of children between the ages of 4 and 10 years seen for MRI scanning at British Columbia's Children's Hospital in Vancouver, Canada, were selected. Twenty NF1 patients from the Vancouver clinic who underwent routine cranial MRI scanning were identified through the National Neurofibromatosis Foundation International Database (NFDB).[17] One of these patients was excluded from the study because he has optic gliomas, which would permit a radiologist to diagnose probable NF1 and might, therefore, bias the recognition of UBOs. The control group consisted of 19 children without NF1 who immediately followed each of the NF1 patients in the radiology logbook, had a head MRI scan for other reasons, and whose age matched within 1 year of that of the corresponding NF1 patient. Six of the 19 control patients had undergone radiation therapy for tumors, six had seizures, two had developmental delay, three had metabolic abnormalities, one had a cortical malformation, and one had postinfectious demyelination.

We used axial fast spin-echo T2-weighted images, 3,555/16/80/21/192 × 256/5 mm/1 mm/2 (repetition time/echo time/echo time/field of view/matrix/thickness/gap/average). The 38 sets of images were examined independently by two experienced pediatric neuroradiologists. The studies were read in a random order, and the radiologists were unaware of the age or diagnosis of the patients. The presence or absence of UBOs and their locations were recorded for each patient.

The sensitivity and specificity of UBOs for NF1 were calculated by using standard formulas.[18] Concordance between the radiologists' interpretations was calculated using the κ statistic.[19]

A second group of NF1 patients was identified through the NFDB. These patients include both probands and affected relatives between the ages of 2 and 21 years who were diagnosed with NF1 by the NIH diagnostic criteria on their first visit to any participating NF clinic. Patients who had not received an ophthalmologic examination with a slit lamp to look for Lisch nodules were excluded. The 657 patients who had cranial MRI examinations were selected from this group, and the presence or absence of UBOs in each patient was determined. The frequency of UBOs was plotted against age in 1-year intervals. We also plotted the Bayesian-corrected proportion of patients by age who met two or more of the current NIH diagnostic criteria (i.e., the proportion of patients in whom NF1 can be diagnosed), and the Bayesian-corrected proportion of patients at each age who would meet two or more diagnostic criteria if UBOs were added as an additional criterion. The 95% confidence intervals of the frequency were calculated by using the method described by Zar.[20]

Table 1 *Frequency and agreement in identification of unidentified bright objects (UBOs) in various anatomic regions of the brain on MRI scans of 19 children with neurofibromatosis 1 (NF1) and 19 age-matched control subjects*

	No. subjects in whom UBOs were identified				
	Radiologist 1		Radiologist 2		
Structure	NF1	Controls	NF1	Controls	Kappa
Cerebellum	13	1	12	1	0.71
Brainstem (total)	17	1	12	1	0.73
Medulla	6	1	6	1	
Pons	14	1	7	1	
Midbrain	7	1	6	0	
Basal ganglia (total)	15	0	17	2	0.79
Caudate	0	0	1	0	
Thalami	11	0	13	1	
Lentiform nuclei	13	0	13	1	
Internal capsule	3	0	8	0	0.49
Cerebrum (total)	2	2	3	4	0.27
Frontal lobe	0	0	1	1	
Temporal lobe	0	1	0	0	
Parietal lobe	2	2	3	3	
Occipital lobe	0	1	0	0	

Results. Cranial MRI examinations on 19 NF1 patients and 19 age-matched controls who did not have NF1 were reviewed blindly by two experienced pediatric neuroradiologists. The NF1 patients had a mean age of 7.9 years. Ten were boys and nine were girls. The control patients had a mean age of 7.8 years. Eleven were boys and eight were girls.

Radiologist 1 identified UBOs in 18 (95%) of the 19 NF1 patients and in three (16%) of the 19 control subjects. Radiologist 2 identified UBOs in all 19 NF1 patients and in five (26%) of 19 control subjects. The five control subjects in whom Radiologist 2 identified UBOs included the three in whom Radiologist 1 identified UBOs.

The sensitivity of UBOs identified by Radiologist 1 for NF1 in this study was 95%. The specificity was 84%. The sensitivity of UBOs identified by Radiologist 2 for NF1 was 100%, and the specificity was 74%.

The κ statistics were calculated to estimate the agreement in identification of UBOs between the two radiologists. The overall agreement was 84% (95% CI, 67% to 100%). Table 1 shows the frequency of identification of UBOs in various anatomic regions of the brain on MRI

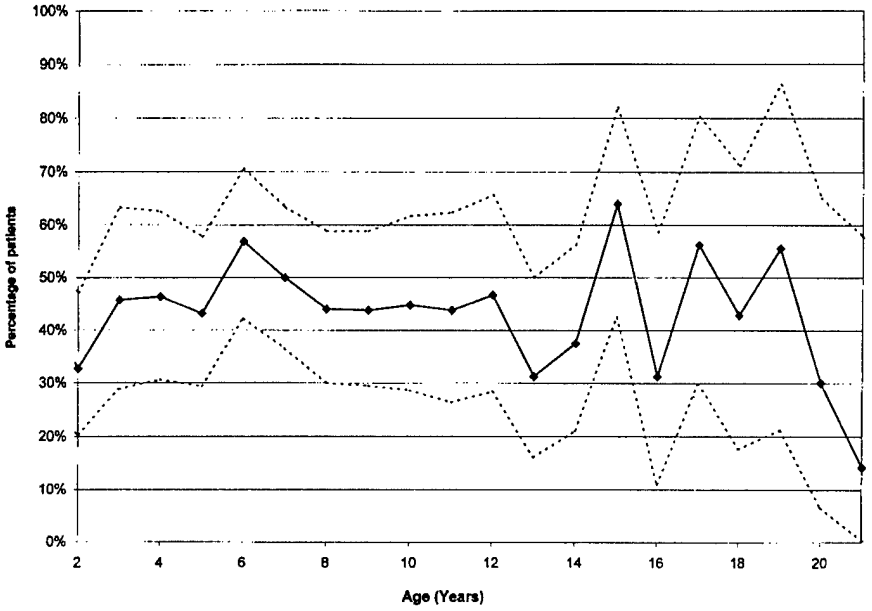

Figure 1. Frequency of unidentified bright objects (UBOs) by age among 657 neurofibromatosis-1 (NF1) patients from the National Neurofibromatosis Foundation International Database who had cranial MRI examinations. Dotted lines indicate 95% confidence intervals.

scans of the children with NF1 and their age-matched controls. Agreement in identification of UBOs between the two radiologists varied from 29% to 79% in various brain regions.

Most of the UBOs in NF1 patients were observed in the cerebellum, brainstem, and basal ganglia. Most of the UBOs in controls were observed in the cerebrum. We therefore recalculated our results, considering only the cerebellum, brainstem, and basal ganglia. Radiologist 1 identified UBOs in these regions in 18 (95%) of the 19 NF1 patients and in 2 (11%) of the 19 control subjects. Radiologist 2 identified UBOs in these regions in 18 (95%) of the 19 NF1 patients and in 4 (21%) of 19 control subjects. The four control subjects in whom Radiologist 2 identified UBOs included the two in whom Radiologist 1 identified UBOs.

The sensitivity of UBOs identified in these regions by Radiologist 1 for NF1 was 95%. The specificity was 89%. The sensitivity of UBOs identified by Radiologist 2 for NF1 was 95%, and the specificity was 80%. The overall agreement when only these three regions were considered was 89% (95% CI, 75% to 100%).

Figure 1 shows the frequency of UBOs by age among 657 NF1 patients from the NFDB who had cranial MRI scans. All brain regions were considered in this patient group. The frequency of UBOs did not vary greatly with age among these patients, all of whom were between 2 and 21 years old. UBOs were diagnosed in ~50% of these pediatric patients with NF1.

Figure 2 shows the proportion of these patients by age who have two or more of the clinical features included in the current NIH diagnostic criteria. The figure also shows the effect of adding UBOs as a diagnostic criterion. For example, at the age of 2 years, 67% of NF1 patients can be diagnosed by using the current NIH diagnostic criteria; 76% have two or more of the features necessary to permit diagnosis if UBOs are added as a diagnostic criterion. The value of including UBOs decreases as patients grow older

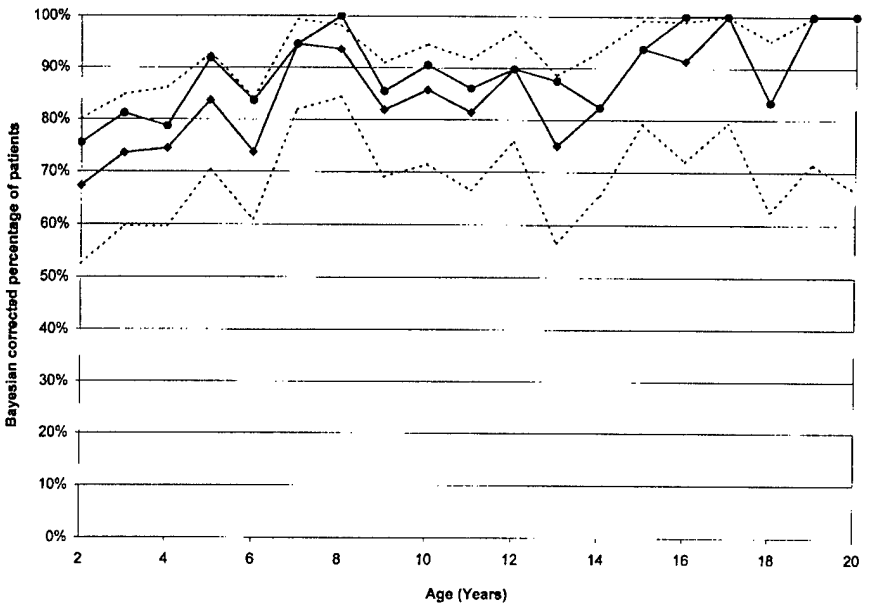

Figure 2. Age at which 657 neurofibromatosis-1 (NF1) patients between ages 2 and 21 years can be diagnosed by meeting two or more diagnostic criteria. ◆, Frequency using the current NIH diagnostic criteria.[1,2] Dotted lines indicate 95% confidence intervals. ●, Frequency with unidentified bright objects (UBOs) as an additional criterion.

Table 2 Frequency of unidentified bright objects (UBOs) in NF1 patients 2 to 20 years old by number of existing NIH diagnostic criteria

	Number of NIH diagnostic criteria						
UBOs	1	2	3	4	5	6	Total
Patients without	43	108	105	53	14	0	323
Patients with (%)	18 (30)	81 (43)	124 (54)	80 (60)	28 (67)	3 (100)	334 (51)
Total	61	189	229	133	42	3	657

$\chi^2 = 28.7$; $p = 0.000027$.

because a larger proportion of them meet the existing NIH criteria.

Table 2 shows the frequency of UBOs in these patients by number of existing NIH diagnostic criteria. UBOs were found in 30% of patients with one of the existing NIH criteria, 43% of patients with two of the criteria, 54% of patients with three, 60% with four, 67% with five, and 100% with six. These differences are almost certainly not due to chance alone ($\chi^2 = 28.7$; $p = 0.000027$).

Discussion. UBOs were identified by MRI examination in all or almost all of the 19 NF1 patients studied between the ages of 4 and 10 years and also were identified in some of the age-matched children who do not have NF1. The relatively high frequency of UBOs among children without NF1 was surprising, but the fact that UBOs were identified independently in three of these 19 subjects by *both* radiologists indicates that UBOs may occur in children with CNS disorders other than NF1 who undergo MRI examination.

We do not routinely obtain cranial MRI examinations on NF1 patients, so our subjects may be more likely to have CNS pathology than an unselected group. The frequency of UBOs in the 19 patients reported here is higher than that found in most other studies, in which it varies between 43% and 93%.[9,10] Similarly, the control group does not represent the general population because MRI scans are performed only on children suspected of having CNS pathology. Eleven of the 19 control patients had disorders associated with white matter changes that might be classified as UBOs on MRI scan. Three of the five controls in whom UBOs were found had such pathology—two had received irradiation for tumors and one had postinfectious demyelination. A recent MRI study found UBOs in 8 (0.8%) of 1,000 healthy individuals, with none observed in those aged 4 to 10 years.[21] It is likely, therefore, that our study underestimates the specificity of UBOs for NF1.

Most of the UBOs in controls were observed in the cerebrum (table 1). Demyelination and irradiation more commonly affect the cerebral hemispheres, perhaps because they are embryologically newer and more sensitive. Most UBOs in subjects with NF1 were observed in the cerebellum, brainstem, or basal ganglia. Griffiths et al.[10] also found that the latter three regions were the most common locations of UBOs in NF1. Furthermore, most of the UBOs in a large series of healthy individuals were found outside these three regions.[21] The distribution of UBOs appears to be different in patients with NF1 than in those who are unaffected by NF1. When only the cerebellum, brainstem, and basal ganglia were considered in our subjects, average specificity increased to 84%. The latter is probably still an underestimate, because two of the four controls in whom UBOs were observed in these regions had CNS pathology that might be classified as UBOs on MRI scan—one had received irradiation for a tumor, and one had postinfectious demyelination.

The agreement in identification of UBOs between these two experienced pediatric neuroradiologists varied considerably among brain regions (see table 1). Agreement would be even lower if comparisons were made between the diagnosis of individual UBOs at various locations within these regions. This inconsistency between radiologists is not surprising, given the subjectivity of MRI, and suggests that UBOs are often difficult to identify with certainty.

Adding UBOs as a diagnostic criterion would permit diagnosis of 30% of young patients with *NF1* mutations who do not fulfill the existing NIH criteria for NF1 (see figure 2). Head MRI examination for UBOs in young patients suspected of having NF1 who do not meet the NIH criteria would reveal asymptomatic optic gliomas in ~10% of NF1 patients.[22] This finding would confirm the diagnosis even if UBOs are not seen. Therefore, the overall improvement in diagnosis would be greater than that estimated in figure 2. However, NF1 patients are not treated for optic glioma before the tumors become symptomatic, and the discovery of an asymptomatic optic glioma may result in considerable and needless worry for the family.

UBOs are relatively uncommon in patients with few of the NIH criteria (see table 2). The frequency is higher in patients who meet more of the criteria. This explains why only 30% of undiagnosed patients were given a diagnosis of NF1 after UBOs were added to the NIH criteria—based on figure 1, we expected this proportion to be ~50%. More importantly, this suggests that UBOs are associated with NF1 severity. UBOs also may be associated with cognitive deficits in NF1.[12]

Adding UBOs as a diagnostic criterion had little effect on the ability to diagnose NF1 in older children. In patients younger than 2 years, areas of increased intensity on T2-weighted images (UBOs) are

difficult to see because incomplete myelination of the brain results in an increased T2-weighted signal from the gray matter.[23] Therefore, the presence of UBOs is unlikely to be a reliable diagnostic tool in children younger than 2 years.

UBOs may be more reliable for diagnosis of NF1 in young children if only the cerebellum, brainstem, and basal ganglia are considered. The lack of consistency with which UBOs were identified suggests that better characterization of these lesions by size or number also may provide better diagnostic precision for NF1 than the simple presence of such lesions.

Any benefit of an earlier diagnosis must be weighed against the anesthetic risk, cost, and anxiety MRI produces in young patients and their families. However, young children who meet only one of the existing NIH diagnostic criteria should be followed up clinically as if they had NF1.

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References

1. Stumpf DA, Alksne JF, Annegers JF, et al. Neurofibromatosis: conference statement. Arch Neurol 1988;45:575–578.
2. Gutmann DH, Alysworth A, Carey JC, et al. The diagnostic evaluation and multidisciplinary management of neurofibromatosis 1 and neurofibromatosis 2. JAMA 1997;278:51–57.
3. Wolkenstein P, Frèche B, Zeller J, Revuz J. Usefulness of screening investigations in neurofibromatosis type 1. Arch Dermatol 1996;132:1333–1336.
4. DeBella K, Szudek J, Friedman JM. Use of the NIH criteria for diagnosis of NF1 in children. Pediatrics 2000 (in press).
5. Aoki S, Barkovich AJ, Nishimura K, et al. Neurofibromatosis types 1 and 2: cranial MR findings. Radiology 1989;272:527–534.
6. Truhan AP, Filipek PA. Magnetic resonance imaging: its role in the neuroradiologic evaluation of neurofibromatosis, tuberous sclerosis, and Sturge-Weber syndrome. Arch Dermatol 1993;129:219–226.
7. Sevick RJ, Barkovich AJ, Edwards MSB, Koch T, Berg B, Lempert T. Evolution of white matter lesions in neurofibromatosis type 1: MR findings. AJR Am J Roentgenol 1992;159: 171–175.
8. DiPaolo DP, Zimmerman RA, Rorke LB, Zackai EH, Bilaniuk LT, Yachnis AT. Neurofibromatosis type 1: pathologic substrate of high-signal-intensity foci in the brain. Radiology 1995;195:721–724.
9. Bognanno JR, Edwards MK, Lee TA, Dunn DW, Roos KL, Klatte EC. Cranial MR imaging in neurofibromatosis. AJR Am J Roentgenol 1988;151:381–388.
10. Griffiths PD, Blaser S, Mukonoweshuro W, Armstrong D, Milo-Mason G, Cheung S. Neurofibromatosis bright objects in children with neurofibromatosis type 1: a proliferative potential? Pediatrics 1999;104:e49.
11. DiMario FJ, Ramsby G. Magnetic resonance imaging lesion analysis in neurofibromatosis type 1. Arch Neurol 1998;55: 500–505.
12. North K. Cognitive function and academic performance. In: Friedman JM, Gutmann DH, MacCollin M, Riccardi V, eds. Neurofibromatosis: phenotype, natural history, and pathogenesis. 3rd ed. Baltimore: Johns Hopkins University Press, 1999:162–189.
13. Curless RG, Siatkowski M, Glaser JS, Shatz NJ. MRI diagnosis of NF-1 in children without café-au-lait skin lesions. Pediatr Neurol 1998;18:269–271.
14. Cnossen MH, Smit FJ, de Goede-Bolder A, et al. Diagnostic delay in neurofibromatosis type 1. Eur J Pediatr 1997;156: 482–487.
15. Kertesz A, Black SE, Tokar G, Benke T, Carr T, Nicholson L. Periventricular and subcortical hyperintensities on magnetic resonance imaging: "rims, caps and unidentified bright objects." Arch Neurol 1988;45:404–408.
16. Levine R, Robbins JA, Maser A. Periventricular white matter changes and oropharyngeal swallowing in normal individuals. Dysphagia 1992;7:142–147.
17. Friedman JM, Birch P, Greene C, NNFFIDB participants. National Neurofibromatosis Foundation international database. Am J Med Genet 1993;45:88–91.
18. Fletcher RH, Fletcher SW, Wagner EH. Clinical epidemiology: the essentials. 3rd ed. Baltimore: Williams & Wilkins, 1996.
19. Fisher LD, Van Belle G. Biostatistics: a methodology for the health sciences. New York: John Wiley and Sons, 1993.
20. Zar JH. Biostatistical analysis. 4th ed. Upper Saddle River: Prentice Hall, 1999.
21. Katzman GL, Dagher AP, Patronas NJ. Incidental findings on brain magnetic resonance imaging from 1000 asymptomatic volunteers. JAMA 1999;282:36–39.
22. Lewis RA, Gerson LP, Axelson KA, Riccardi VM, Whitford RP. von Recklinghausen neurofibromatosis. II. Incidence of optic gliomata. Ophthalmology 1984;91:929–935.
23. Ballesteros MC, Hansen PE, Soila K. MR imaging of the developing human brain. Part 2. Postnatal development. Radiographics 1993;13:611–612.

The Use of Unidentified Bright Objects on MRI for Diagnosis of Neurofibromatosis 1 in Children. *K. DeBella[1], K. Poskitt[2], J. Szudek[1], J.M. Friedman[1].* 1) Medical Genetics, UBC, Vancouver, BC, Canada; 2) Radiology, BC Children's Hospital, Vancouver, BC, Canada.

Unidentified Bright Objects (UBOs) have been observed on cranial magnetic resonance imaging (MRI) in 45-80% of children with neurofibromatosis 1 (NF1). Because the NIH Diagnostic Criteria often do not permit unequivocal identification of NF1 in young children, UBOs have been proposed as an additional diagnostic criterion. We examined the sensitivity and specificity of UBOs for NF1 in 19 affected children and 19 age-matched controls. We measured the agreement of UBO recognition between two experienced pediatric neuroradiologists who independently examined the MRI studies on these patients. We also used data from 858 NF1 patients between the age of 2 and 21 from the NNFFIDB (NNFF International Database) who had cranial MRI scans to determine whether the addition of UBOs to the existing NIH diagnostic criteria would improve early diagnosis.

The overall sensitivity of UBOs for NF1 averaged 97%, and the specificity averaged 79%. Agreement between the two radiologists was 84% overall (95% C.I. 67-100%). We do not routinely obtain cranial MRI examinations on NF1 patients, so our subjects as well as our controls are more likely to have central nervous system pathology than unselected children. Adding UBOs as a diagnostic criterion would permit 85% rather than 80% of two-year old NF1 patients from the NNFFIDB to be diagnosed, a difference that is not statistically significant. UBOs occur in children who do not have NF1, but the frequency and clinical significance in normal children need to be defined. UBOs may be more reliable for diagnosis of NF1 if they can be better characterized and differentiated from similar lesions that occur in other patients. Our data suggest that requiring a patient to have three or more UBOs in the cerebellum, medulla, pons, midbrain, thalami or lentiform nuclei is a more specific criterion for NF1 than the simple presence of such lesions.

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Use of the National Institutes of Health Criteria for Diagnosis of Neurofibromatosis 1 in Children

Kimberly DeBella; Jacek Szudek; and Jan Marshall Friedman, MD, PhD

ABSTRACT. *Objective.* The National Institutes of Health (NIH) Diagnostic Criteria for neurofibromatosis 1 (NF1) are very useful clinically, but some individuals who are later shown to have NF1 cannot be diagnosed in early childhood using these criteria. The aim of this study is to determine the value of the NIH Diagnostic Criteria for NF1 in early childhood, to determine the age at which diagnosis can confidently be made, and to clarify the age at onset of the cardinal clinical features used in the NIH Diagnostic Criteria.

Methods. We studied 1893 NF1 patients under 21 years old from the National Neurofibromatosis Foundation International Database to determine the age at which the features included in the NIH Diagnostic Criteria appear.

Results. Approximately 46% of sporadic NF1 cases fail to meet the NIH Diagnostic Criteria by 1 year of age. Nearly all (97%; 95% confidence interval: 94–98) NF1 patients meet the criteria for diagnosis by 8 years old, and all do so by 20 years old. The usual order of appearance of the clinical features listed as NIH criteria is café-au-lait macules, axillary freckling, Lisch nodules, and neurofibromas. Symptomatic optic glioma is usually diagnosed by 3 years old, and characteristic osseous lesions are usually apparent within the first year of life.

Conclusion. The diagnosis of NF1 cannot always be made in young children using the NIH Diagnostic Criteria. Modification of these criteria may be necessary for children under 8 years old. *Pediatrics* 2000;105:608–614; *neurofibromatosis 1, National Institutes of Health Diagnostic Criteria, natural history.*

ABBREVIATIONS. NF1, neurofibromatosis 1; NIH, National Institutes of Health.

Neurofibromatosis 1 (NF1) is a progressive multisystem disorder. This autosomal dominant disease affects between 1/2000 and 1/4500 people.[1,2] Half of all NF1 cases are familial, and half are caused by new mutations.[2–5] NF1 is diagnosed using a set of clinical criteria developed by a National Institutes of Health (NIH) Consensus Conference in 1988[6] and recently reaffirmed by another group of experts.[7] To meet the NIH Diagnostic Criteria for NF1, an individual must demonstrate at least 2 of the 7 features listed in Table 1.

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The NIH Diagnostic Criteria for NF1 are thought to be very reliable in distinguishing adults with and without NF1 on the basis routine clinical and ophthalmological examinations.[7,8] The reliability of these criteria for diagnosing NF1 in children has not been rigorously assessed, but some NF1 patients clearly cannot be diagnosed in early childhood by the NIH Diagnostic Criteria.[9,10]

Earlier diagnosis of NF1 may be beneficial to both affected children and their families. Genetic counseling could be offered to parents and other relatives earlier, and interventions for learning or developmental problems could be initiated sooner.[11] Diagnosis of NF1 may also be important to implement supportive care and to allow earlier detection of serious complications.

We used clinical information from the National Neurofibromatosis Foundation International Database[12] to assess the value of the NIH Diagnostic Criteria in children.

METHODS

Three groups of patients were selected from the National Neurofibromatosis Foundation International Database for study. The first group consisted of 1402 patients, including both probands and affected relatives, under 21 years old who were diagnosed as having NF1 by the NIH Diagnostic Criteria on their first visit to a participating neurofibromatosis clinic. Patients who had not received an ophthalmological examination with a slit lamp were excluded. For purposes of classifying café-au-lait spots, we defined prepubertal as <13 years old and postpubertal as 13 years or older. The number of cardinal clinical features that contribute to the NIH criteria present in each patient was determined and plotted against age in 3-year intervals.

A second study group consisted of 39 NF1 patients from the National Neurofibromatosis Foundation International Database who did not meet the NIH criteria for diagnosis on their first recorded examination but who did so on a subsequent examination. Only patients who were reexamined at periodic intervals of 4 years or less were included in this group.

Finally, we analyzed the age-specific prevalence rates for individual cardinal clinical features in each of 1893 NF1 patients under 21 years old who were diagnosed as having NF1 on their first examination at a participating neurofibromatosis clinic. This group of patients includes the 1402 patients in the first group as well as patients who met the NIH criteria for NF1 but who have not had a slit lamp examination. The total number of patients included for each cardinal clinical feature varies because we excluded cases in which the presence or absence of the particular feature could not be determined unequivocally from available data.

The 95% confidence intervals were calculated for all data using the method for proportions described by Zar.[13] This method uses the relationship between an F distribution and a binomial distribution to compute a confidence interval for the binomial parameter.

RESULTS

The frequencies by age of 2, 3, 4, or 5 or more of the clinical features that comprise the NIH Diagnostic Criteria among 1402 patients with NF1 are shown in Fig 1. The patients in this analysis include both sporadic cases and those who have inherited an NF1 mutation from an affected parent. Some familial cases are included even if they only exhibit 1 cardinal clinical feature because the NIH Diagnostic Criteria permit a diagnosis of NF1 for patients who have 1 such feature and a positive family history.

Almost all these NF1 patients (97%; 95% confidence interval: 94–98) have 2 or more of the cardinal clinical features included in the NIH Diagnostic Criteria by 8 years old. All the NF1 patients have 2 or more cardinal clinical features by 20 years old. In contrast, 30% of NF1 patients under 1 year of age have only 1 of the cardinal clinical features. These infants must all have an affected first degree relative to be diagnosed with NF1.

All the patients included in Fig 1 were diagnosed with NF1 on their first visit to a participating neurofibromatosis clinic. We also studied 39 NF1 patients with an average age of 1.6 years who did not meet the NIH Diagnostic Criteria on first examination but were diagnosed as having NF1 by these criteria at a later date. The ages of these patients when they were found to meet the NIH Diagnostic Criteria are shown in Fig 2. These patients were found to have at least 2 of the cardinal clinical features at an average of 4.6 years of age, with a maximum age of 19. These findings support our observation in Fig 1 that nearly all NF1 patients meet the NIH Diagnostic Criteria by 8 years old, and all patients do so by 20 years old.

The progressive nature of NF1 results in an increased frequency of many of the cardinal clinical features with age. To determine the usual sequence of appearance of the cardinal clinical features, we plotted the prevalence of each feature among 1893 NF1 patients (probands and affected relatives) under 21 years old. The ages at which these patients met the NIH criterion for café-au-lait macules is given in Fig 3, for neurofibromas in Fig 4, for inguinal or axillary freckling in Fig 5, for Lisch nodules in Fig 6, for optic glioma in Fig 7, and for distinctive osseous lesions in Fig 8.

Ninety-nine percent of NF1 patients have 6 or more café-au-lait macules greater than 5 mm in di-

TABLE 1: NIH Diagnostic Criteria for NF1

Cardinal Clinical Features (Any Two or More Are Required for Diagnosis)[1]
6 or more café-au-lait macules over 5 mm in greatest diameter in prepubertal individuals and over 15 mm in greatest diameter in postpubertal individuals
2 or more neurofibromas of any type or 1 plexiform neurofibroma
Freckling in the axillary or inguinal regions
Optic glioma
2 or more Lisch nodules (iris harmartomas)
A distinctive osseous lesion such as sphenoid dysplasia or thinning of the long bone cortex with or without pseudarthrosis
A first degree relative (parent, sibling, or offspring) with NF1 by the above criteria

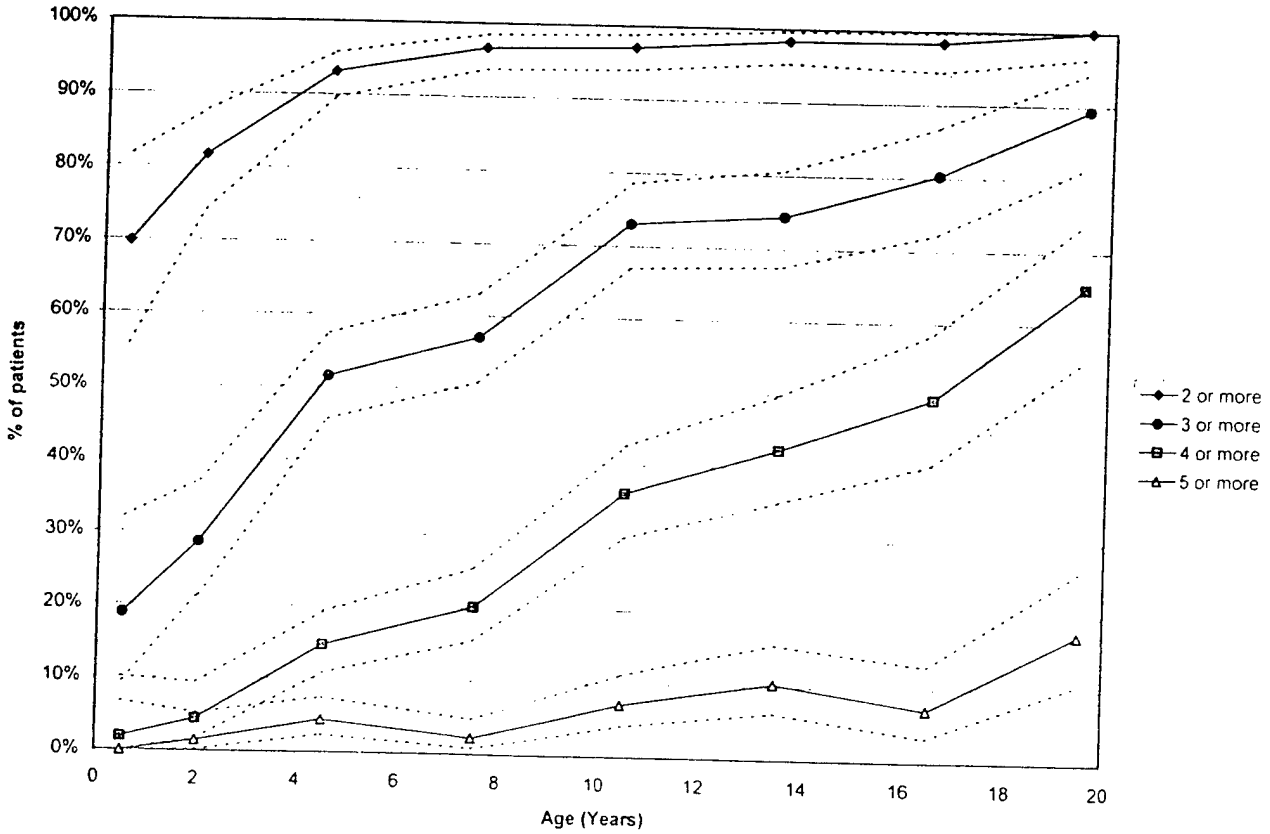

Fig 1. Age-specific frequency of 2, 3, 4, or 5 or more clinical features included in the National Institutes of Health Diagnostic Criteria among 1402 NF1 patients under 21 years old (including 95% confidence intervals).

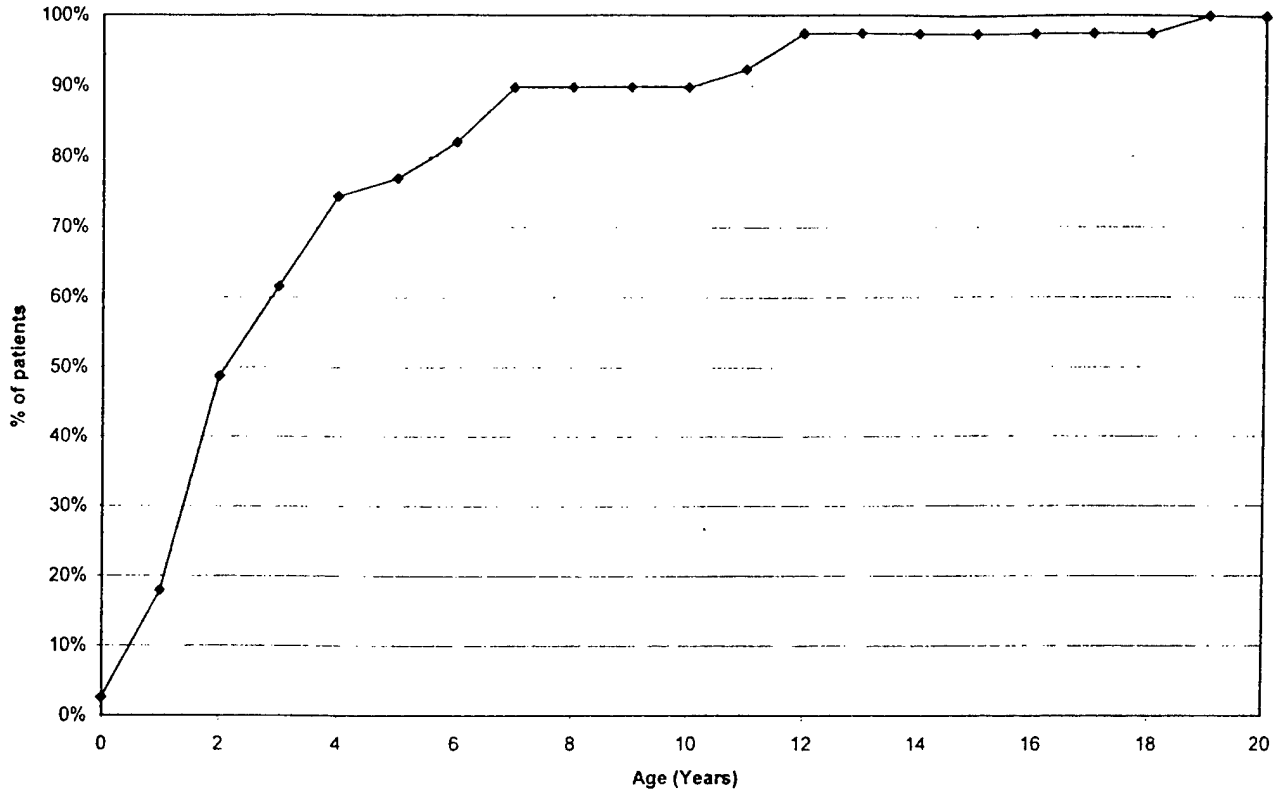

Fig 2. Age at which 39 NF1 patients meet 2 or more National Institutes of Health Diagnostic Criteria.

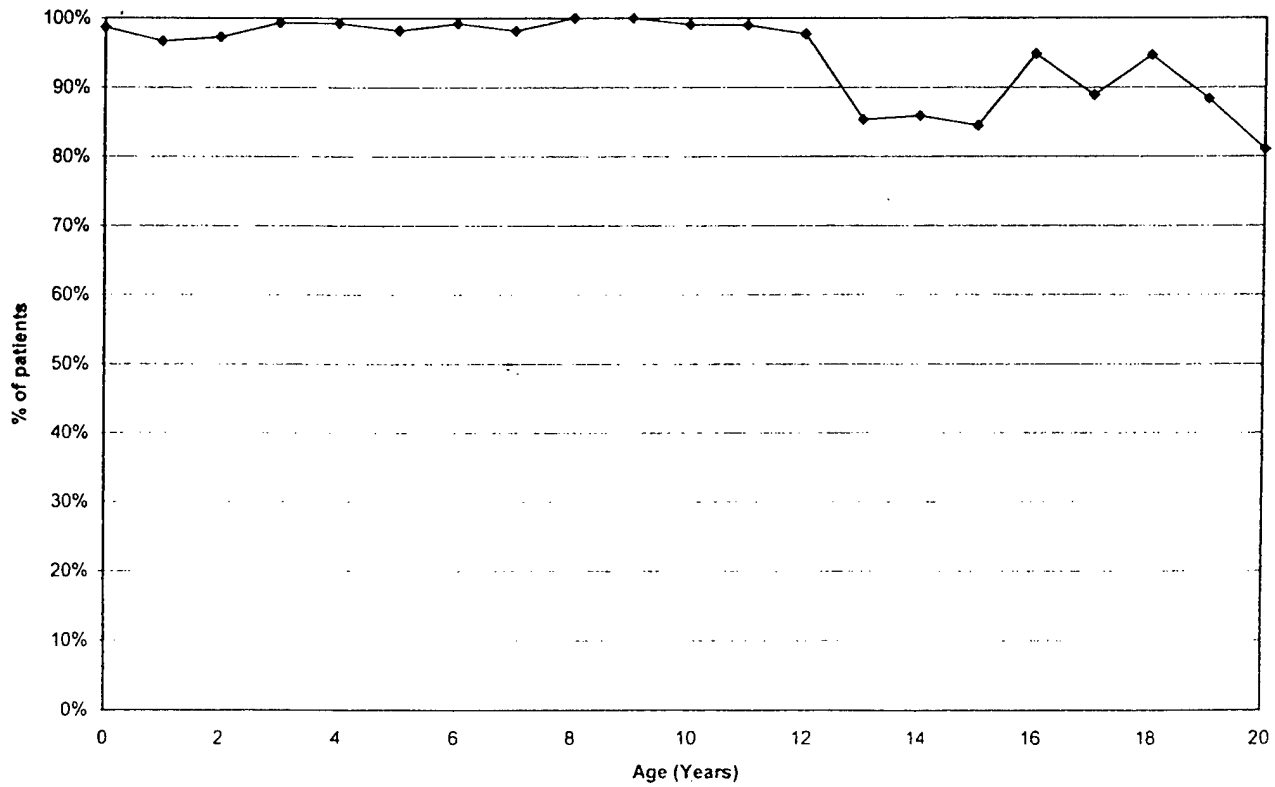

Fig 3. Age at which 1888 NF1 patients have café-au-lait spots.

ameter by 1 year of age. Inguinal or axillary freckling affects a maximum of 90% of NF1 patients by 7 years old. Lisch nodules affect >70% of patients by 10 years old. Neurofibromas are present in 48% of 10-year-old patients and 84% of 20-year-old patients. Symptomatic optic glioma is diagnosed in the first year of life in 1% of NF1 patients and reaches maximum frequency (~4%) by 3 years old. Characteristic osseous lesions are usually apparent within the first year of life and occur in ~14% of NF1 patients.

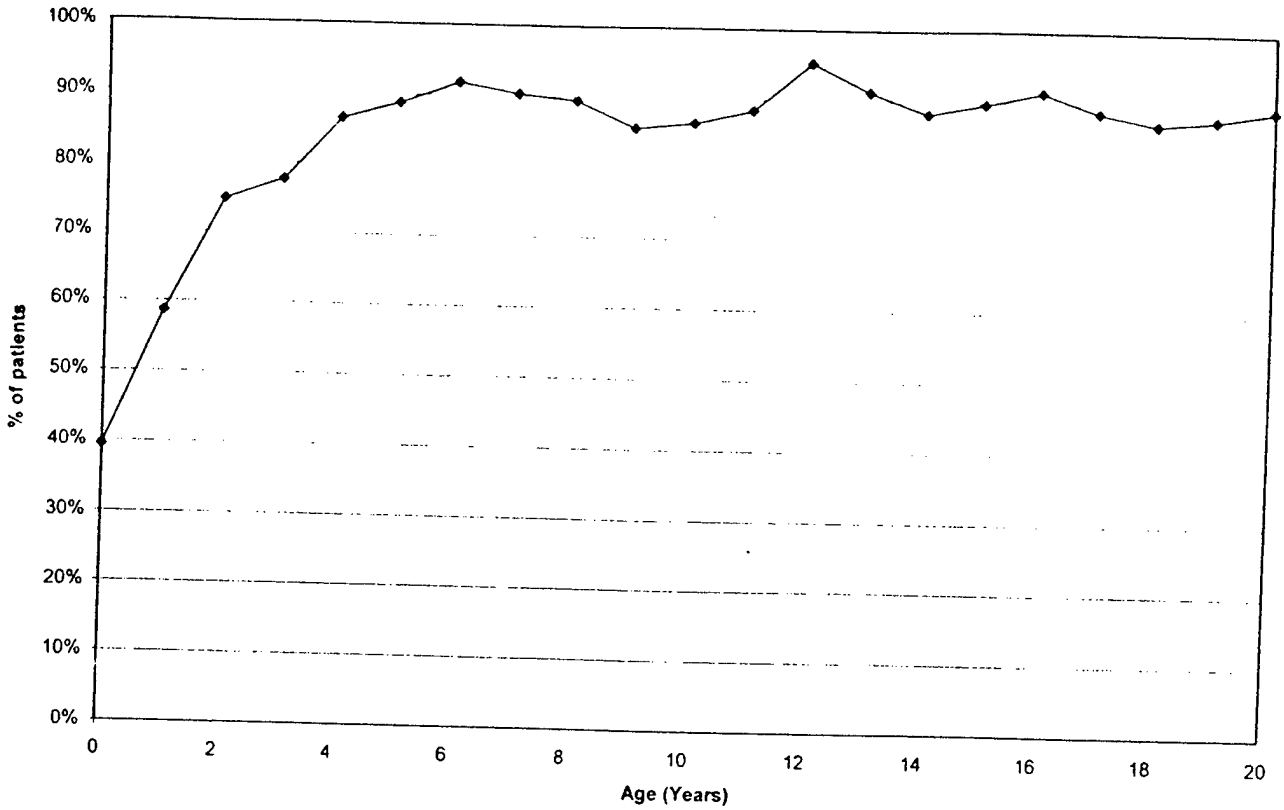

Fig 4. Age at which 1835 NF1 patients have inguinal or axillary freckling.

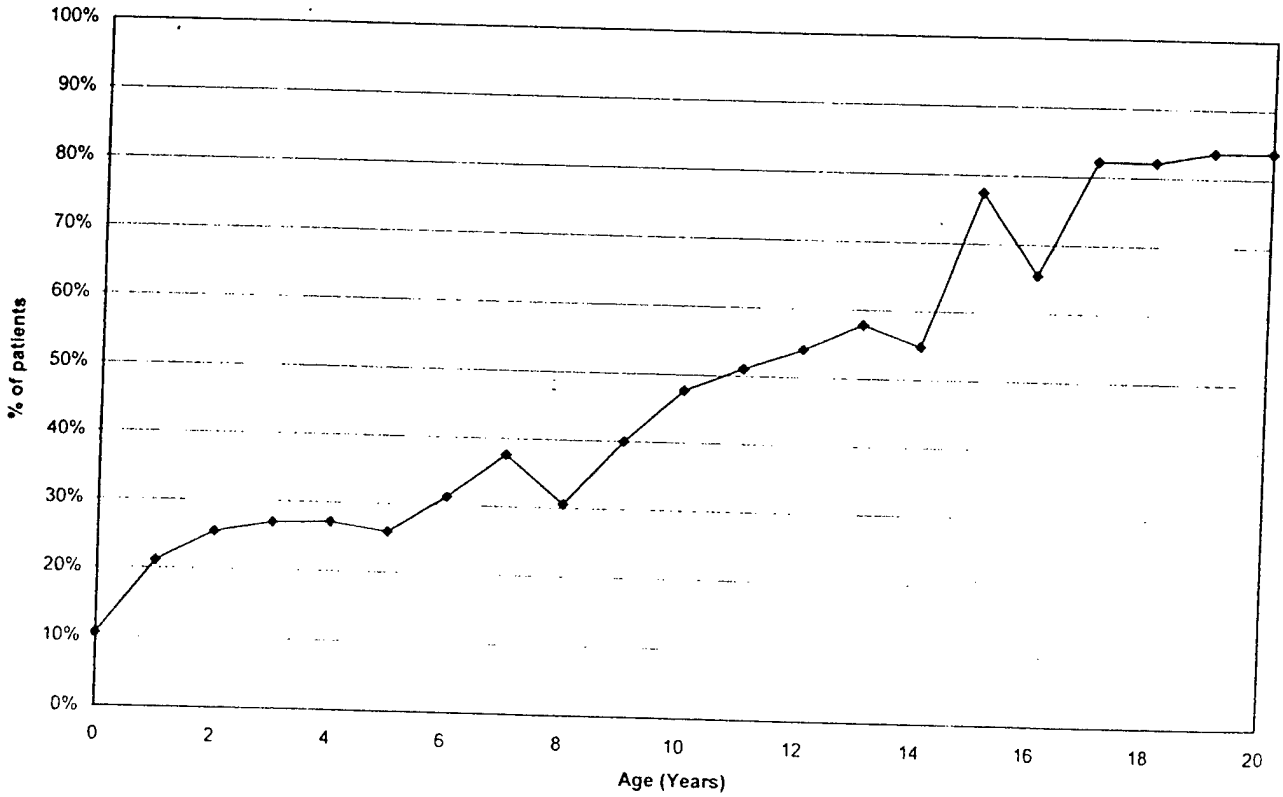

Fig 5. Age at which 1891 NF1 patients have 2 or more neurofibromas of any type or 1 plexiform neurofibroma.

DISCUSSION

The number of cardinal clinical features included in the NIH Diagnostic Criteria increases in NF1 patients with age. As shown in Fig 1, 70% of NF1 patients can be diagnosed as having the disease before they are 1 year old based on 2 or more of the cardinal clinical features. The other 30% of NF1 patients under 1 year of age have only 1 of the cardinal clinical features. These children must all have an affected first degree relative to be diagnosed with

Fig 6. Age at which 1402 NF1 patients have 2 or more Lisch nodules.

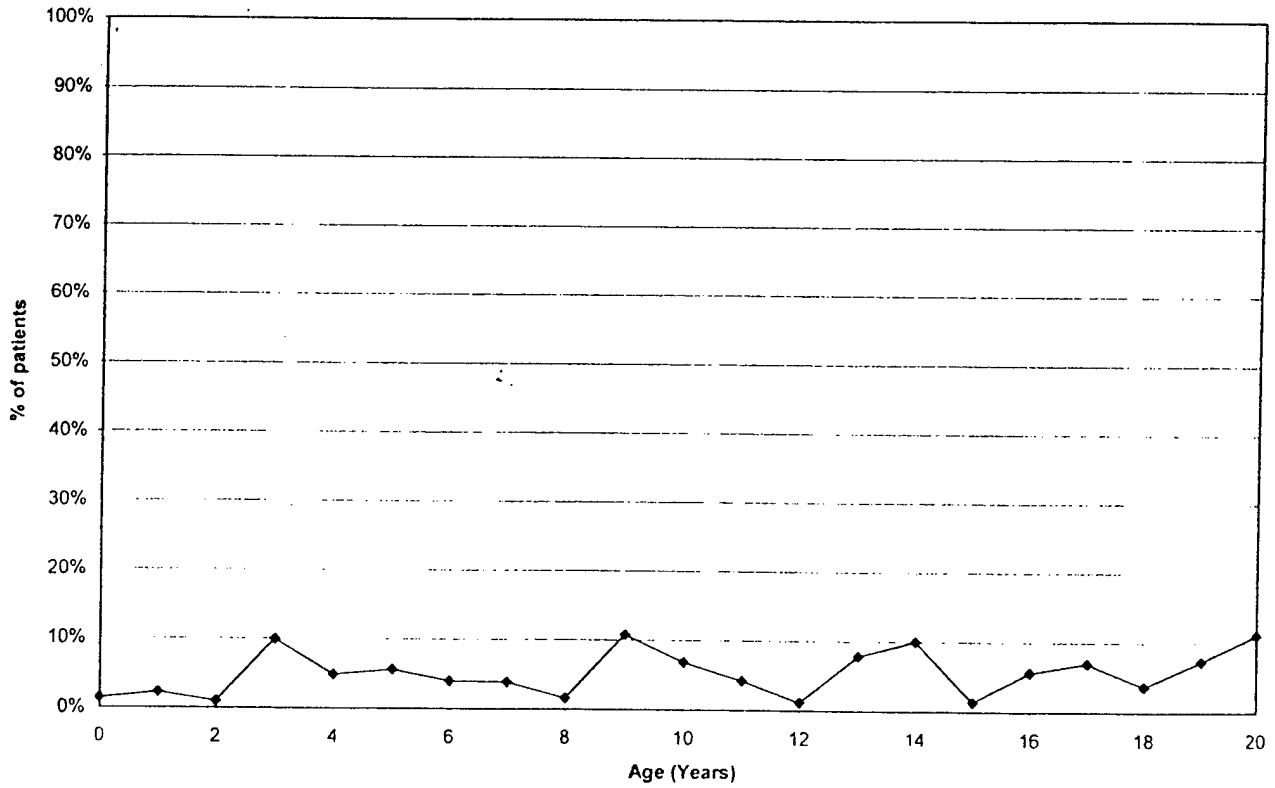

Fig 7. Age at which 1807 NF1 patients have symptomatic optic glioma.

NF1 according to the NIH criteria. We would expect an equal number of sporadic cases to have only 1 cardinal feature by 1 year of age because approximately half of all NF1 patients represent sporadic cases.[2-4] These sporadic cases would not be diagnosed as having NF1 before 1 year of age because they do not meet the NIH Diagnostic Criteria.

If the total number of NF1 patients diagnosed by 1 year of age is *N*, the number of sporadic cases that remain undiagnosed by age 1 is .3 *N*. The total num-

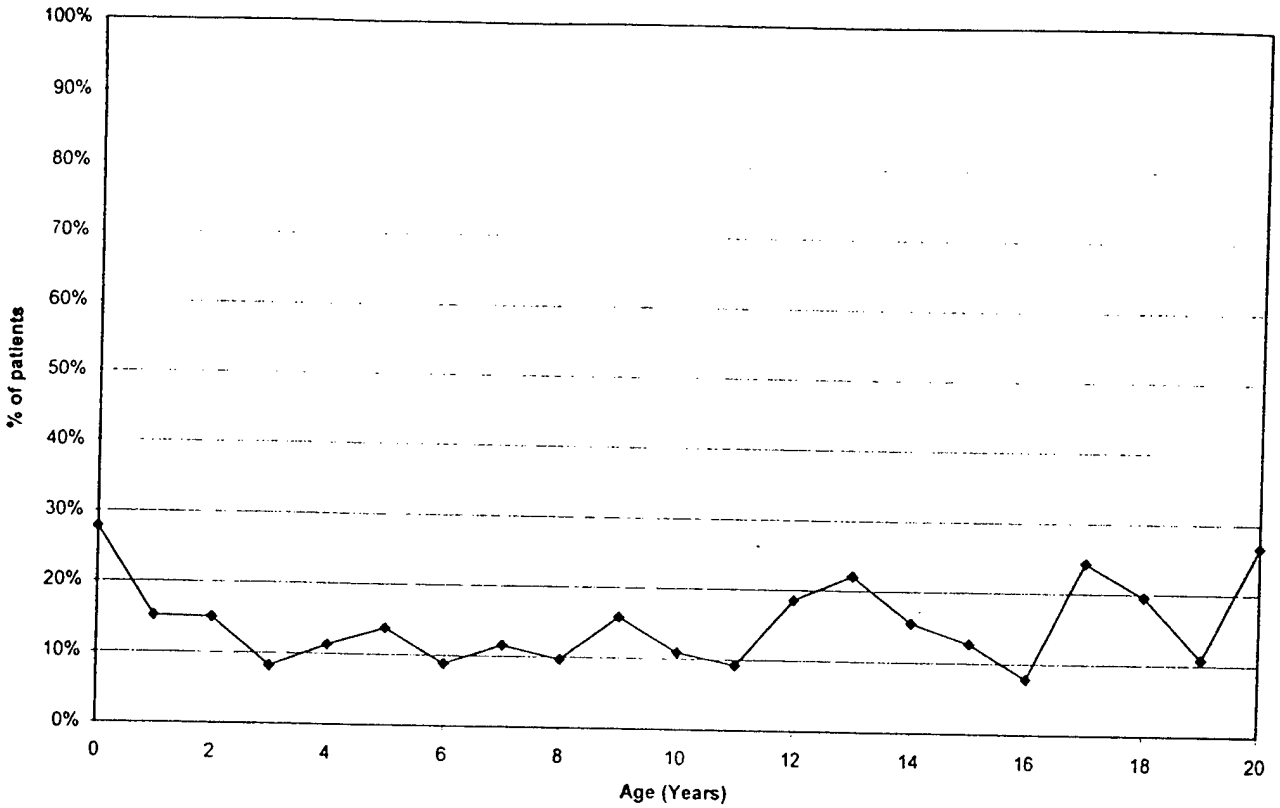

Fig 8. Age at which 1722 NF1 patients have a distinctive osseous lesion.

ber of individuals with NF1 mutations at 1 year of age is (N + .3 N), half of whom represent sporadic cases. The proportion of sporadic NF1 cases that remain undiagnosed by 1 year of age is, therefore,

$$\frac{0.3\,N}{1/2(N + .3N)} = 46\%.$$

From this, we estimate that ~46% of sporadic cases lack 2 or more of the cardinal clinical features and cannot be diagnosed by the NIH Diagnostic Criteria by 1 year of age. NF1 probably cannot be diagnosed using these criteria in at least 5% of the sporadic cases by 8 years old. All NF1 probands have at least 2 of the cardinal clinical features included in the NIH Diagnostic Criteria by 20 years old.

The age-specific prevalence rates of the individual cardinal clinical features that comprise the NIH Diagnostic Criteria are displayed in Figs 3 to 8. The usual order of appearance of these features is café-au-lait macules, axillary freckling, Lisch nodules, and neurofibromas. Optic glioma is usually diagnosed within the first 3 years of life, and characteristic osseous lesions within the first year.

Café-au-lait macules, which are present within the first year of life in most NF1 patients, are not likely to develop after 4 years old if not already present. Inguinal or axillary freckling is commonly present by 7 years old and does not usually develop after this age. Lisch nodules usually develop before 10 years of age. Neurofibromas are usually present by 20 years old if they are going to develop.

The frequencies of the individual cardinal clinical features seen in this study are similar to those previously reported.[1,3,14,15] The data used for these analyses are cross-sectional in nature and are, therefore, not ideal for examining progression of features. A more accurate representation of NF1 patients could be obtained from a large longitudinal study, but only limited longitudinal data are available.

The diagnosis of NF1 cannot always be made or ruled out with confidence in young children using the NIH Diagnostic Criteria. Diagnosis of NF1 can confidently be made using these criteria by 8 years old in most children and by 20 years old in all children. However, we recommend that young children who have 6 or more café-au-lait spots greater than 5 mm in largest diameter but do not have any of the other cardinal clinical features be followed as if they had NF1. Almost all these children will develop other clinical manifestations of NF1 with time.[9,10]

Modification of the NIH Diagnostic Criteria may be necessary to provide more reliable diagnosis for young children. The inclusion of other clinical features that are both sensitive and specific for NF1 in pediatric patients may improve diagnosis. Short stature,[16] macrocephaly,[16] and unidentified bright objects on head magnetic resonance imaging[17–19] have been suggested as potentially useful in this regard. Routine radiographic screening for common osseous lesions, such as pseudarthrosis, sphenoid wing dysplasia, and dysplastic vertebrae, should also be considered. We are currently studying the value of adding these features to the NIH Diagnostic Criteria.

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REFERENCES

1. Huson SM, Hughes RAC. *The Neurofibromatoses*. London, UK: Chapman and Hall; 1994;1.3.2:9
2. Littler M, Morton NE. Segregation analysis of peripheral neurofibromatosis (NF1). *J Med Genet*. 1990;27:307–310
3. Riccardi VM. *Neurofibromatosis*. 2nd ed. Baltimore, MD: Johns Hopkins University Press; 1992:328–342
4. Clementi M, Barbujani G, Turolla L, Tenconi R. Neurofibromatosis-1: a maximum likelihood estimation of mutation rate. *Hum Genet*. 1990;84: 116–118
5. North K. Neurofibromatosis type 1: review of the first 200 patients in an Australian clinic. *J Child Neurol*. 1993;8:395–402
6. Stumpf DA, Alksne JF, Annegers JF, et al. Neurofibromatosis: conference statement. *Arch Neurol*. 1988;45:575–578
7. Gutmann DH, Aylsworth A, Carey JC, et al. The diagnostic evaluation and multidisciplinary management of neurofibromatosis 1 and neurofibromatosis 2. *JAMA*. 1997;278:51–57
8. Wolkenstein P, Frèche B, Zeller J, Revuz J. Usefulness of screening investigations in neurofibromatosis type 1. *Arch Dermatol*. 1996;132: 1333–1336
9. Obringer AC, Meadows AT, Zackai EH. The diagnosis of neurofibromatosis-1 in the child under the age of 6 years. *Am J Dis Child*. 1989; 143:717–719
10. Korf BR. Diagnostic outcome in children with multiple café au lait spots. *Pediatrics*. 1992;90:924–927
11. Cnossen MH, Smit FJ, de Goede-Bolder A, Frets PG, Duivenvoorden HJ, Niermeijer MF. Diagnostic delay in neurofibromatosis type 1. *Eur J Pediatr*. 1997;156:482–487
12. Friedman JM, Birch P, Greene C, National Neurofibromatosis Foundation International Database participants. National Neurofibromatosis Foundation International Database. *Am J Med Genet*. 1993;45:88–91
13. Zar JH. *Biostatistical Analysis*. Englewood Cliffs, NJ: Prentice Hall; 1984: 378–379
14. Carey JC, Laub JM, Hall BD. Penetrance and variability in neurofibromatosis: a genetic study of 60 families. *Birth Defects: Original Article Series*. 1979;5B:271–281
15. Samuelsson B, Axelsson R. Neurofibromatosis: a clinical and genetic study of 96 cases in Gothenburg, Sweden. *Acta Derm Venereol*. 1981; 95(suppl):67–71
16. Cnossen MH, Moons KGM, Garssen MPJ, et al. Minor disease features in neurofibromatosis type 1 (NF1) and their possible value in diagnosis of NF1 in children ≤6 years and clinically suspected of having NF1. *J Med Genet*. 1998;35:624–627
17. Curless RG, Siatkowski M, Glaser JS, Shatz NJ. MRI Diagnosis of NF-1 in children without café-au-lait skin lesions. *Pediatr Neurol*. 1998;18:269–271
18. Goldstein SM, Curless RG, Donovan Post MJ, Quencer RM. A new sign of neurofibromatosis on magnetic resonance imaging of children. *Arch Neurol*. 1989;46:1222–1224
19. DiMario FJ, Ramsby G, Greenstein R, et al. Neurofibromatosis type 1: magnetic resonance imaging findings. *J Child Neurol*. 1993;8:32–39

Use of NIH Criteria for Diagnosis of Neurofibromatosis Type 1 in Children.

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The NIH Diagnostic Criteria for Neurofibromatosis Type 1 (NF1) [Stumpf et al. Arch Neurol 1988;45:575-578; Gutmann et al. JAMA 1997; 278: 51-57] have proven to be extremely useful clinically. The diagnosis of NF1 can reliably be made or excluded in adults on the basis of a routine clinical and ophthalmological examination using these criteria. However, some individuals who are later shown to have NF1 cannot be diagnosed in early childhood by means of the NIH criteria. We studied 1893 individuals under the age of 21 with NF1 from the National Neurofibromatosis Foundation International Database to determine the age at which the features included in the NIH Diagnostic Criteria appear.

Almost all NF1 probands meet two or more diagnostic criteria (98%; 0.95 CI = 96-100%) by 8 years of age, but we estimate that at least 4% of the sporadic cases do not meet the 2 criteria necessary for diagnosis at this age. 75% of NF1 probands have 2 criteria by the age of 1 year; a minimum of 50% of sporadic cases do not meet the 2 criteria necessary for diagnosis by the age of 1. All NF1 probands meet two diagnostic criteria by 20 years old.

99% of NF1 patients have café au lait macules before 1 year of age. Inguinal or axillary freckling reaches a maximum frequency of 90% of NF1 patients by 7 years old. Lisch nodules affect a maximum of 73% of patients by age 10. Neurofibromas are present in 48% of 10 year olds and 84% of 20 year old patients. Symptomatic optic glioma is diagnosed in the first year of life in 1% of NF1 patients and reaches its maximum frequency (about 4%) by the age of 3 years. Characteristic osseous lesions are usually apparent within the first year of life and occur in about 14% of NF1 patients. The usual order of appearance of the NIH criteria in children with NF1 is café au lait macules, axillary freckling, Lisch nodules, and neurofibromas.

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Short communication

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Patricia Birch

An Association Between Optic Glioma and Other Tumours of the Central Nervous System in Neurofibromatosis Type 1

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Abstract

Neurofibromatosis type 1 (NF1) has a very heterogeneous phenotype. It is not currently possible to predict which patients will have mild disease and which will develop serious complications. Medical management of patients with NF1 might be improved if subgroups of patients who are at especially high (or low) risk for particular complications could be identified.

We have begun an analysis of NF1 patients in the National Neurofibromatosis Foundation International Database (NNFFID) to identify possible associations between the occurrence of clinical features. A striking association has been observed between the presence of optic glioma and of other central nervous system (CNS) tumours in NF1 patients. This association is not dependent on the effect of age. No association is seen between optic glioma and non-CNS neoplasms. The association of optic glioma and other intracranial neoplasms in patients with NF1 suggests that there are fundamental pathophysiological differences between patients with and without optic glioma.

Key words: NF1 – Optic glioma – CNS tumours

Introduction

The dominantly-inherited condition, neurofibromatosis type 1 (NF1), is extremely variable clinically (3, 4). NF1 is progressive throughout life, but the rate of progression may differ greatly from year to year. Various affected members of a single family, all of whom presumably have the same mutant NF1 allele, often have very different disease manifestations and complications.

We do not know if each individual with NF1 is equally susceptible to all manifestations of the condition or if some patients are at much higher risk for certain associated problems. This uncertainty is one of the greatest burdens of NF1 for many families. Most serious NF1 complications, such as malignancy, are infrequent. This makes it difficult to recognize patterns of clinical features that might characterize patients who are at unusually high risk to develop such serious complications.

We are analyzing the detailed clinical information available on over 2500 cases of NF1 from the multi-centre National Neurofibromatosis Foundation International Database (NNFFID) to look for patterns of clinical associations among various NF1 features. We have observed a striking association between the occurrence of optic glioma and other intracranial neoplasms in patients with NF1.

Material and methods

This analysis was limited to 684 unrelated probands with NF1 in the NNFFID who have had intracranial imaging studies. Affected relatives were excluded from the analysis to eliminate associations that might result from intrafamilial correlations. Statistical significance was calculated using a chi-square (X^2) test with *Yates'* correction for continuity.

Results

The group of 154 (22%) of the 684 probands had optic glioma. There is insufficient information recorded in the database to describe the exact location of the optic gliomas, but over half of them (n = 86) were asymptomatic. Among the 154 patients with optic glioma, 17 (11.0%) had a second CNS tumour and 4 (2.6%) had a non-CNS neoplasm (other than neurofibromas). Among the 530 patients who have no evidence of optic glioma on intracranial imaging study, 8 (1.5%) have a CNS tumour and 20 (3.8%) have a non-CNS neoplasm. The neoplasms that occurred in these patients are listed in Table 1.

There is a strong association between presence of optic glioma and other CNS tumours ($X^2 = 28$, $p = 0.000001$, odds ratio = 8.1; 95% CI = 3.2–22.1) but no association between optic glioma and non-CNS neoplasms.

Among the 154 patients with optic glioma, 3 of 17 people with a CNS tumour and 1 of 4 people with a non-CNS neoplasm had received radiation therapy, a potential cause of secondary tumours. If these patients are removed from the analysis, the association between the presence of optic glioma and another CNS tumour is still highly significant ($X^2 = 18$, $p = 0.00002$, odds ratio = 6.7; 95% CI = 2.5–18.7).

In NF1, optic glioma rarely, if ever, develops after the first decade of life (2), but 57% of the patients in the study were over 10 years old at the time of cranial imaging. In order to minimize any confounding effect of age on the association between optic glioma and other CNS tumours, we stratified our sample by age: We examined the subset of patients who were under 10 years old at the time of their first cranial imaging study. In this young group of 297 patients the association between optic glioma and other CNS

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Tumours reported in 154 probands with optic glioma		Tumours reported in 530 probands with no optic glioma	
CNS tumours N = 17	non-CNS neoplasms N = 4	CNS tumours N = 8	non-CNS neoplasms N = 20
*Glioma – 15 *Astrocytoma – 2	Schwannoma – 2 Rhabdomyosarcoma – 1 Sarcoma – 1	*Glioma – 6 *Astrocytoma – 1 Meningioma – 1	Schwannoma – 7 Neuroblastoma – 3 Sarcoma – 2 *Wilm*'s tumour – 1 Lymphoma – 1 Carcinoma – 3 Ganglioneuroma – 1 Fibromyoma – 1 Pheochromocytoma – 1

*We have used the clinicians' descriptions of these glial tumours: Some clinicians have used the terms "astrocytoma" and "glioma" interchangeably.

Table 1 Tumour types in 684 probands receiving intracranial imaging and numbers of individuals with each type of tumour (as reported to the NNFF International Database).

tumours was still highly significant ($X^2 = 15$, $p = 0.00009$, odds ratio = 24.5; 95% CI = 3.2–1090). Moreover, we found no significant difference, using *Student*'s t-test, between the mean age of patients with and without other CNS tumours (5.3 and 5.7 years) or those with or without optic gliomas (5.9 and 5.8 years).

Discussion

In 1994, *Kuenzle* et al (1) observed 11 additional "cerebral tumors" among 21 NF1 patients with optic glioma. The second tumours were found, on average, 4 years after diagnosis of the optic glioma. *Kuenzle* and his associates expressed concern about a possible association between the occurrence of optic gliomas and other intracranial neoplasms in NF1 patients. We have analyzed data from a much larger group of patients and confirm the presence of this association.

The overall proportion of patients with optic glioma in our study (22%) is somewhat higher than that previously reported. However, ours is a convenience sample of patients evaluated by specialists at major centres with a particular interest in neurofibromatosis. It would not be surprising to find a higher prevalence of optic glioma among such patients than among a population-based series as a result of referral bias.

It is possible that individuals who are being followed for optic glioma are more likely to be diagnosed with a second CNS tumour because of more frequent or more intensive cranial imaging studies. However, CNS tumours occurred in 13% of 68 patients with symptomatic optic gliomas compared to 9% of 86 patients with asymptomatic optic gliomas. These proportions are not significantly different, suggesting that a bias of observation is unlikely to account for the association.

The association of optic glioma and other intracranial neoplasms in patients with NF1 suggests that there are fundamental pathophysiological differences between such patients and NF1 patients who do not develop optic glioma. These differences might result from specific mutations of the NF1 gene, from the effect of modifying genes, or from non-genetic factors. If modifying genes or specific mutant NF1 alleles are responsible, the association between optic glioma and other intracranial neoplasms that we have observed in NF1 probands should also be present within families. We intend to explore this possibility in future analyses.

Acknowledgements

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References

1 *Kuenzle, Ch., M. Weissert, E. Roulet, H. Bode, S. Schefer, Th. Huisman, K. Landau, E. Boltshauser:* Follow-up of optic gliomas in children with neurofibromatosis type 1. Neuropediatrics 25 (1994) 295–300

2 *Listernick, R., J. Charrow, M. Greenwald, M. Mets:* Natural history of optic pathway tumors in children with neurofibromatosis type 1: A longitudinal study. J. Pediatr. 125 (1994) 63–66

3 *Riccardi, V. M.:* Neurofibromatosis. Phenotype, Natural History and Pathogenesis. 2nd ed., Baltimore. Johns Hopkins University Press (1992)

4 *Rubenstein, A. E., B. R. Korf:* Neurofibromatosis. A Handbook for Patients, Families and Health-Care Professionals. New York, Thieme Medical Publishers (1990)

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Type 1 Neurofibromatosis: A Descriptive Analysis of the Disorder in 1,728 Patients

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Type 1 Neurofibromatosis, NF1, is a common genetic disorder with variable clinical manifestations. Although NF1 often is only of cosmetic concern, serious and even lethal complications may occur. It is not possible to predict which symptoms will develop in any affected individual.

The NNFF International Database is a multicentre collaborative system for collecting information about this condition. At the time of this analysis, complete clinical information was available on 1,479 probands and 249 of their affected relatives with NF1. On average, the age at diagnosis of NF1 was 8 years younger in the probands than in the affected relatives ($P<.01$). Many of the manifestations of NF1 were more frequent in the probands than in their affected relatives. The age-specific prevalence of most manifestations of NF1 increases with age. Despite biases inherent in a convenience sample from specialist clinics, the frequencies of many of the serious manifestations of NF1 are similar to those of two smaller population-based studies. The frequencies in this study are likely representative of patients seen at specialized clinics. Am. J. Med. Genet. 70:138–143, 1997.

KEY WORDS: neurofibromatosis type 1; NF1; database

INTRODUCTION

Neurofibromatosis type 1 (NF1) was described more than 100 years ago [Mulvihill, 1990]. Although the clinical manifestations of NF1 are well known [Crowe et al., 1956; Riccardi, 1992; Rubenstein and Korf, 1990; Carey et al., 1979; Huson, 1994], the course of the condition in individual patients is largely unpredictable. This unpredictability is a major concern for most patients with NF1 and their families.

The NNFF International Database (NNFFID) is a multicentre collaborative system for collecting demographic information, descriptions of signs and symptoms, basic measurements, and certain psychosocial information on individuals and families with NF. The NNFFID was established and is maintained by the National Neurofibromatosis Foundation. Twenty-nine NF clinics throughout the world voluntarily enter data into the NNFFID. Complete data from over 2,500 patients are currently available. Any qualified investigator can access these data for use in appropriate studies by contacting the authors.

We present here a descriptive analysis of data on 1,728 individuals with NF1 contributed to the NNFFID between 1991 and 1995.

METHODS

The structure and technical details of the database were previously described [Friedman et al., 1993]. The database includes 98 data items on each patient and allows for serial data collection on patients who are seen repeatedly in a particular NF clinic. In order to ensure uniformity, every data item is defined in a database dictionary and incoming data are examined at the central database for possible inconsistencies. A numbering scheme employed in the coding allows members of individual families to be identified and their relationship to the proband described.

The information in the NNFF International Database was exported to SYSTAT version 5.0 [1990] for statistical analysis on an industry-standard 80486 personal computer. We used the F-distribution and the binomial distribution to derive 95% upper and lower confidence limits for binary traits and chi-square statistics for comparison of frequencies between groups. Yates' correction was used for small expected frequencies [Zar, 1984].

All patients included in this analysis have NF1 as defined by the NIH Consensus Conference [1988]. We excluded cases described as segmental NF or "other type of NF." Approximately 20% of the data are longitudinal, representing second or subsequent clinic visits of patients upon whom there is already information in

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the database. We have only included information from the most recent clinic visit for this analysis. The most recent visit was chosen to avoid counting individual patients more than once, which would occur if each record rather than each patient were analyzed. In addition, most NF1 features persist once they develop; using the most recent visit therefore provides "cumulative" frequencies of various disease manifestations.

All of the centres that contribute data to the NNFF International Database are specialized clinics with a particular interest in neurofibromatosis. Ascertainment of a patient by an individual NF Clinic depends to some degree on the nature and clinical interests of that clinic. Frequencies of manifestations in probands with NF1 are likely to be affected by this biased ascertainment. We have analyzed data from probands and affected relatives separately on the assumption that data from relatives will exhibit less ascertainment bias and thus more accurately reflect the natural history of NF1.

RESULTS

At the time this analysis was performed, the NNFF International Database contained 1,728 cases diagnosed with NF1. Of these, 1,479 were probands and 249 were affected relatives. The average age of the probands was 16.9 years (range: 0–73 years) at the time of examination; the average age of affected relatives was 22.2 years (range: 0–80 years). Age at diagnosis was 8.2 (range: 0–71 years) and 16.1 (range: 0–78 years) years for probands and relatives, respectively. Both age at exam and age at diagnosis were significantly greater in relatives than probands ($P<.01$).

The probands were 48.3% male and 45.4% of the included relatives were male. Neither of these frequencies differs significantly from the 50% expected value; 83% of the individuals studied are described as Caucasian, 6.6% as Asian, and 3.5% as of African ethnic origin. We found no significant differences in any of our variables across racial backgrounds, although the numbers of non-Caucasians are small.

The overall prevalence of each of the 98 clinical manifestations recorded in the NNFF International Database was calculated. The frequencies of the 25 most common or most important clinical manifestations are shown in Table I.

In addition to manifestations with strict clinical definitions, we collected data on functional and subjective effects of NF1 reported by the patients or their relatives. Table II summarizes some of this information.

Most manifestations of NF1 increase in frequency with age. The age distribution of the 1,479 probands and 249 relatives is shown in Figure 1. Age-specific prevalences for discrete neurofibromas and for plexiform neurofibromas in NF1 probands and affected relatives are presented in Figures 2 and 3. Figure 4 shows the frequency of all tumours (excluding neurofibromas and optic glioma) and of CNS tumours. The numbers and types of tumours reported are summarized in Table III.

We have also examined the progression of NF1 with age by graphing the average number of N.I.H. diagnostic criteria [NIH Consensus Conference, 1988] met by patients in different age groups (Fig. 5). In order to permit comparison of probands and relatives, we have eliminated the criterion of "affected first degree relative" in the data used for this figure.

TABLE I. Prevalence of Clinical Manifestations of NF1 in 1,479 Probands and 249 Affected Relatives*

Clinical manifestation	Prevalence in probands (%) n	Prevalence in affected relatives (%) n	
Six or more café-au-lait macules	90.7 (1,479)	78.3 (249)	**
Intertriginous freckling	85.5 (1,418)	76.7 (249)	**
Discrete neurofibromas	53.6 (1,479)	55.8 (249)	
Subcutaneous	36.6 (1,456)	34.4 (241)	
Cutaneous	37.9 (1,460)	44.0 (243)	
Pendulous	5.6 (1,467)	14.3 (238)	**
One plexiform neurofibroma	24.1 (1,479)	15.7 (249)	**
Two or more plexiform neurofibromas	5.3 (1,475)	2.0 (247)	**
Hemangiomas	6.5 (1,455)	6.2 (241)	
Xanthogranulomas	2.5 (1,458)	2.5 (241)	
Lisch nodules	57.0 (1,054)	69.9 (183)	**
Symptomatic optic glioma	4.3 (1,415)	1.3 (235)	*
Sensorineural hearing loss	5.3 (1,445)	5.4 (240)	
Seizures	6.4 (1,479)	2.4 (249)	**
Hydrocephalus	4.0 (1,460)	2.0 (246)	
Congenital heart defect	2.1 (1,467)	1.2 (245)	
Hypertension	4.2 (1,460)	3.7 (242)	
Precocious puberty	3.5 (600)	1.4 (69)	
Pseudarthrosis	2.2 (1,465)	0.8 (243)	
Congenitally bowed tibia without pseudarthrosis	3.8 (1,479)	1.6 (245)	
Scoliosis	26.0 (1,380)	13.6 (212)	**
Dysplastic sphenoid wing	11.3 (256)	2.5 (40)	
Facial asymmetry	8.0 (1,471)	3.3 (245)	**
Possible Noonan phenotype	3.7 (1,439)	1.6 (244)	
Total neoplasms (excluding neurofibromas and optic gliomas)	4.9 (1,479)	3.2 (249)	
CNS neoplasms (excluding neurofibromas and optic gliomas)	2.0 (1,479)	1.2 (249)	

*Asterisks indicate manifestations in which the frequency in the probands differs significantly from that in relatives: *indicates $P < .05$; **indicates $P < .01$. The number of individuals on whom the calculation is based is given in brackets beside each percentage. When the number is less than the number of individuals in the study this is because of responses coded as "unknown." For example, Lisch nodules require a slit lamp exam to meet the NNFF International Database criterion for presence or absence. Individuals who have not had a slit lamp exam are coded as "unknown" and excluded from this table.

DISCUSSION

The differences in age at examination and age at diagnosis between the probands and their relatives in this study probably reflect the fact that most of the cases were submitted to the NNFF International Database by clinics located in pediatric hospitals. Differences in the prevalence of NF1 complications between

TABLE II. Prevalence of Functional and Subjective Effects of NF1 in 1,479 Probands and 249 Relatives[a]

Functional or subjective effect	Prevalence in probands (%) n	Prevalence in relatives (%) n
Troublesome itching	2.8 (1,479)	1.6 (245)
Educational difficulty	40.0 (562)	36.6 (71)
Cosmetic problems from NF	40.1 (1,310)	31.1 (222) *
Psychological or social problems from NF	40.1 (1,248)	26.5 (211) **
Surgery related to NF in last 12 months	12.1 (1,303)	4.9 (225) **

[a]Asterisks indicate manifestations in which the frequency in the probands differs significantly from that in relatives: *indicates $P < .05$; **indicates $P < .01$.

probands and their relatives may be influenced by this difference in age.

The overall prevalences of various NF1 manifestations in this analysis are similar to those reported by five other authors [Riccardi, 1992; Crowe et al., 1956; Carey et al., 1979; Huson et al., 1989; Samuelsson et al., 1981] (Table IV). Another study, by North [1993], was omitted because of a complete overlap of her patients with those of NNFFID. There is no known overlap with any of the other studies. In comparison to other studies, the sample included in the NNFFID is most similar in patient selection and size to Riccardi's: over half of NNFFID patients were referred from other specialty clinics. The studies of Huson and Samuelsson are population based whereas Carey's patients were referred to a general genetics clinic. The majority of patients in Crowe's study were ascertained via hospital records. Thus, the results of this study are most generalizable to specialist neurofibromatosis clinics.

Apart from differences in ascertainment, precise comparisons amongst the different studies are difficult for four reasons:

1) The frequencies of most features of NF1 change with age, and the age distribution of the studies probably differs. Specific age data were not available for all of the studies.
2) Features are defined differently in different studies.

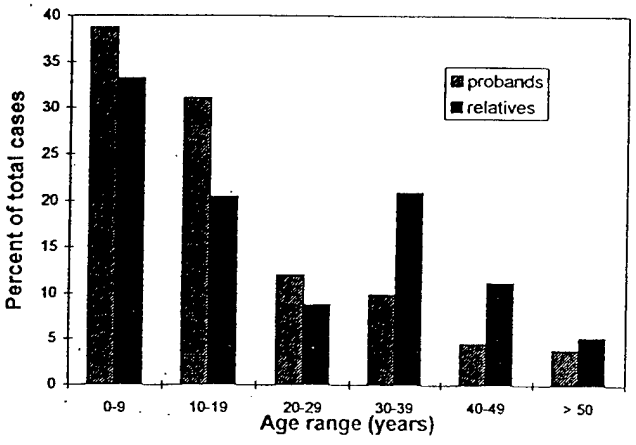

Fig. 1. Age distribution of 1,479 probands and 249 relatives shown as a proportion of the total number of probands and relatives, respectively.

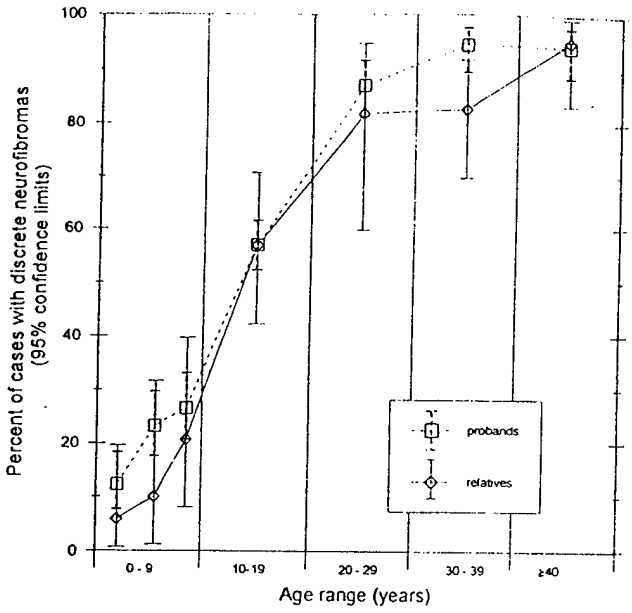

Fig. 2. Age-specific prevalence of discrete neurofibromas in probands and relatives.

Neurofibromas are a particular problem: our study uses mobility within the skin to differentiate between discrete neurofibromas that are cutaneous (moves with the skin) or subcutaneous (does not move with the skin). This definition was used by Crowe et al. (1956) and by Riccardi (1992), who also distinguishes between "nodular and plexiform" neurofibromas. Huson et al. (1994) use the terms "dermal, nodular, and plexiform" to describe neurofibromas.

3) Differing criteria were used in the studies for identification of various manifestations. For example,

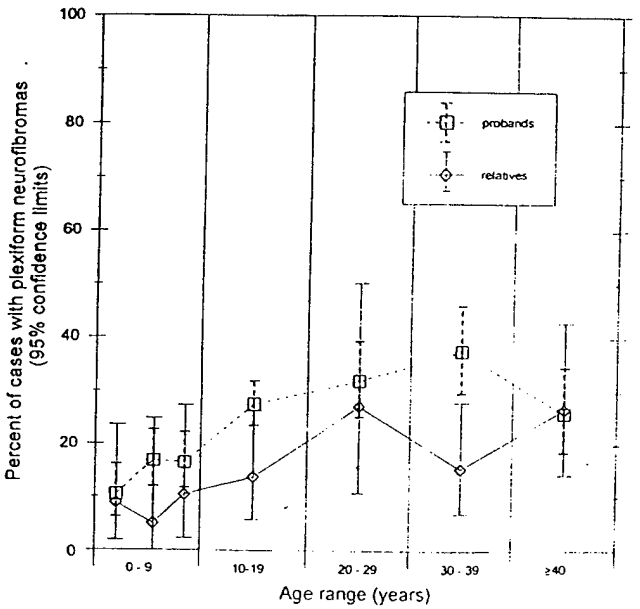

Fig. 3. Age-specific prevalence of plexiform neurofibromas in probands and relatives.

TABLE III. Summary of Types of Neoplasms Reported by Age

Tumour type	Number of tumours Age range (years) 0–9 n = 583	10–19 n = 511	20–29 n = 200	30–39 n = 198	≥40 n = 164	Total
Glioma	16	8	2	5	2	33
CNS tumour-type unknown	2		2			4
Adenoma					1	1
Carcinoma	1			2	5	8
Lymphoma	1			2		3
Fibromyoma				1		1
Melanoma					1	1
Neuroblastoma	1					1
Pheochromocytoma					2	2
Rhabdomyoma	1					1
Sarcoma		1	6	1	2	10
Schwannoma	2		6	1	6	15
Wilms	1					1
Total neoplasms	25	9	16	12	19	81

Riccardi makes routine use of MRI to diagnose plexiform neurofibromas, whereas our study does not require this criterion for diagnosis. If use of MRI substantially improves the identification of plexiform neurofibromas, one would expect a higher frequency in Riccardi's study than in ours. In the subjective findings of Table II, this is a particular problem. For example, "troublesome itching" was seen in only 2.6% of patients, much lower than the 5–10% figure cited by Riccardi [p 58] but this may be due to the NNFFID's definition: "troublesome itching occurring more than once per week." If all individuals with pruritus are included, the NNFFID figure jumps to 10%.

4) Most previous studies are too small to permit adequate estimation of the frequencies of rarer complications of NF1. Even with the large number of patients included in our study, the 95% confidence intervals of our estimates are quite wide for uncommon complications, especially when the patients are stratified by age.

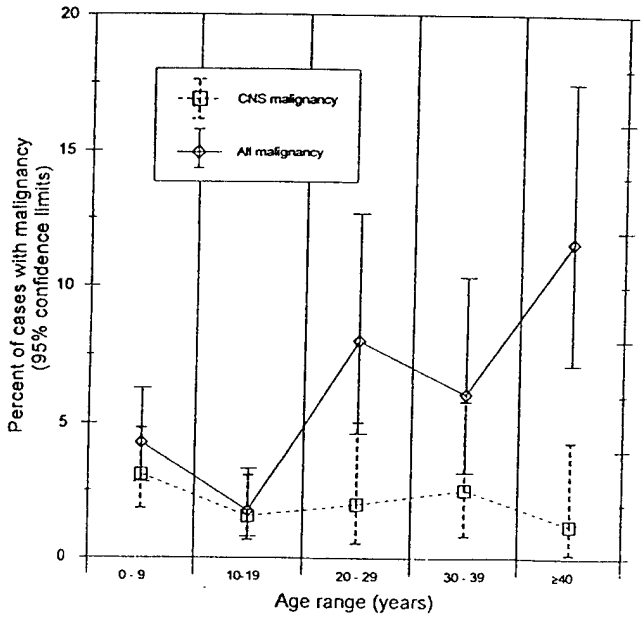

Fig. 4. Age-specific prevalence of malignancy.

Probands tend to be more severely affected than relatives in our study. This is evident for individual signs such as plexiform neurofibromas (Fig. 3), for which the frequency in probands is higher than or equal to that in relatives in all age groups although the differences are not statistically significant. It is also true for the overall number of N.I.H. criteria: at almost all ages, the probands meet more N.I.H criteria than their relatives do (Fig. 5), although the differences are only statistically significant in children under 4 years. These differences are probably a result of ascertainment bias: more severely affected individuals are more likely to come to medical attention and become the proband of a family.

Scrutiny of our data also shows that certain features that should always be coded as present once they have occurred, such as plexiform neurofibromas and pseudarthrosis, appear to decrease in frequency in middle age. This could be due to ascertainment bias or to coding error: a corrected case of pseudarthrosis could be erroneously coded as "pseudarthrosis absent" in an adult. Nevertheless, it is likely that the frequencies presented here for younger probands are representative of patients seen at specialized pediatric NF clinics.

A number of specific individual findings of this study are worthy of comment: The incidence of xanthogranulomas is approximately 2% in our study as well as in other reported series. There has been some concern regarding a possible association between juvenile xanthogranulomas and chronic myelogenous leukemia [Zvulunov et al., 1995]. In our study, this association is not observed: 31 individuals under the age of 10 have xanthogranulomas, but none of these children has CML. In the entire NNFF International database, only one patient had CML. This child did have xanthogranulomas, but he was excluded from our analysis because his diagnosis was "probable NF" and not definite NF1, as required for inclusion.

Precocious puberty is also of concern in NF1. It is not

TABLE IV. Comparative Frequency of NF1 Findings in Six Studies*

Finding	Study's first author and total study size					
	Friedman (n = 1728) %	Riccardi (n = 953) %	Crowe (n = 203) %	Carey (n = 131) %	Huson (n = 135) %	Samuelsson (n = 91) %
CAL	89	100	78		84	82
Discrete NFs	54					93
Plexiform NFs	23	40	16		32	
Xanthogranulomas	2	2			1	
Lisch nodules	59	84			85	
Pseudarthrosis	2	3		0	4	
Scoliosis	24	25	16	5	10	≥10
Optic glioma-symptomatic	4			2		0
Seizures	6	6		6	7	3–9
Malignancy	5	4	5			10

*Blank cells in the table indicate that the frequency of a particular anomaly was not reported in that study. Data from all studies include both probands and other affected relatives.

clear whether precocious puberty is always associated with optic glioma, although this usually is the case [Habiby et al., 1995]. The overall incidence of precocious puberty in this study was 3.3%. In the entire database of NF1 patients, 29 individuals have precocious puberty. Of these, 16 have optic glioma (14 of which are clinically symptomatic); of the remainder, 1 has optic nerve thickening, 4 have no evidence of optic glioma clinically or by cranial imaging, and 8 have not been scanned but have no clinical signs of optic glioma. These data are consistent with the interpretation that precocious puberty in NF1 is frequently but not always associated with underlying optic glioma.

Several non-specific features have been reported previously to occur more frequently in NF1 patients than in the general population. The incidence of non-febrile seizures seems relatively consistent across studies, at about 6%. This is approximately double the background pediatric incidence [Hoekelman et al., 1987].

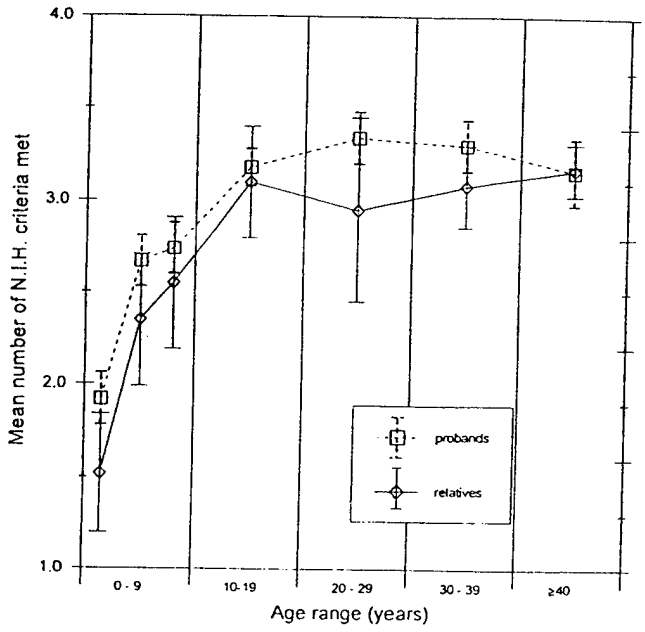

Fig. 5. Mean number of N.I.H. criteria met by probands and relatives at different ages. The criterion of affected first degree relative has been omitted.

Hypertension is widespread in the general adult population but is uncommon in children. The overall prevalence in NNFFID probands of all ages is 4%. In children aged 19 years or less, the prevalence of hypertension is 1.8%, not substantially different from the background pediatric prevalence of about 1–2% [Hughes et al., 1984]. The incidence of congenital heart defects in the NNFFID sample is 2%, whereas the general incidence is approximately 0.8% [Connor et al., 1991].

The prevalences given for most of the clinical manifestations in Table I are based on at least 95% of the probands so that ascertainment bias within the sample is not a concern. A notable exception is sphenoid wing dysplasia. The study prevalence of 11.3% is based on only 256 of the 1,479 probands. This prevalence is much higher than Riccardi's figure of 5% [pp 312] and may well be a result of ascertainment bias, at least in part. Diagnosis of sphenoid wing dysplasia is only possible by skull radiography or intracranial imaging. Patients with facial asymmetry are more likely to receive such studies and may be more likely to have sphenoid wing dysplasia than patients without asymmetry. Riccardi's patients received routine cranial imaging.

The frequencies of most disease manifestations in NNFFID patients are similar to those in Riccardi's database with the exception of a lower incidence of CAL, plexiform neurofibromas, and Lisch nodules in the NNFFID. The difference in CAL likely reflects differences in definition: Riccardi coded CAL as present if any macules were observed, whereas NNFFID requires the presence of 6 CAL to meet the criteria. Plexiform neurofibromas in locations such as the paraspinal areas frequently require imaging for diagnosis, particularly in younger patients. Imaging studies may be responsible for the apparently higher frequency of plexiforms with age in Riccardi's data (see Fig. 3). Such imaging was performed routinely on Riccardi's patients but is not a requirement for patients entered in the NNFFID.

The lower incidence of Lisch nodules in our study may reflect the relatively young age of the NNFFID cohort. The lower prevalence of Lisch nodules our probands (57%) than in their the somewhat older relatives (70%) is consistent with this interpretation. The prevalence of 84% in Riccardi's study is similar to that ob-

served in the population-based study of Huson et al. (85%). This is probably a more accurate lifetime figure.

Our overall incidence of scoliosis of 24% (26% in probands, 14% in relatives) appears high in comparison to all other studies except Riccardi's (25%). We counted all cases of scoliosis, including mild disease, because information on degrees of curve is frequently missing from the NNFFID. Where possible, similar definitions were used to compare frequencies in Table IV. (For example, Carey's prevalence (5%) includes "mild–severe" scoliosis and Huson's (10%) includes individuals "requiring surgery" and those who are "less severe"). The discrepancy is likely due to the ascertainment bias in the NNFFID due to the large number of patients from pediatric specialists' clinics as well as inconsistent definitions of "mild" scoliosis.

Table II reports the prevalence of NF-related surgery within the last year as 12% for probands and 5% for relatives. This figure is much higher than would be expected generally among NF1 patients and probably reflects the ascertainment of most of the probands from specialist clinics in major referral centres. Unfortunately, the reason and the nature of the surgery are not specified in our data.

The prevalence of other functional effects of NF1 also varies across studies. For example "Educational difficulty" ranges from 21% in Carey's cohort to 69% in Riccardi's. NNFFID, Huson's, and Samuelsson's studies fall in between at 40, 38, and 30%, respectively. These differences probably result from different definitions of these problems in various studies.

The frequency of many NF1 symptoms varies with age. The age-dependent incidence of discrete neurofibromas shown in Figure 2 is one of the best examples. The most dramatic increase in neurofibromas occurs between ages 10 and 20, likely coinciding with adolescence. It is noteworthy that about 5% of patients over 40 have no discrete neurofibromas, although they otherwise meet the criteria for NF1.

The second, more serious complication that apparently increases with age is tumours other than neurofibromas (Fig. 4). This figure shows the total number of cases in which any type of tumour (CNS or non-CNS) was diagnosed. Within the 95% confidence intervals, the incidence of CNS tumours appears to remain fairly constant in the 1 to 3% range, whereas the total number of tumours appears to increase after the second decade of life. This increase is due in part to appearance of tumours that may be unrelated to NF. For example, there are at least two cases each of bowel and breast cancer in the group over 40 years. Both are malignancies that are common in the general population. With increasing numbers in the NNFFID, it should soon be possible to determine to what extent the number of non-CNS tumours exceed the background risk.

Despite the bias in ascertainment towards more severely affected individuals, it is evident that the most serious complications of NF1 are all uncommon. This is the case in our study as well as in the two population-based studies [Huson et al., 1989; Samuelsson et al., 1981]. Further studies are now underway to look for associations among various clinical manifestations of NF1. We are also trying to identify specific patterns of traits that may be indicative of a more severe long-term outcome. Better knowledge of the natural history of NF1 may help improve our ability to predict the likelihood of complications of individual patients and their affected relatives.

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REFERENCES

Carey JC, Laub JM, Hall BD (1979): Penetrance and variability in neurofibromatosis: A genetic study of 60 families. New York: Alan R. Liss, Inc., for the National Foundation–March of Dimes. BD:OAS XV (5B): 271–281.

Connor JM, Ferguson-Smith MA (1991): "Essential Medical Genetics." 3rd Ed. Oxford: Blackwell Scientific Publications, pp 198.

Crowe FW, Schull WJ, Neel JV (1956): "A Clinical Pathological and Genetic Study of Multiple Neurofibromatosis." Springfield: Charles C Thomas.

Friedman JM, Birch P, Greene C and the NNFF International Database Participants (1993): National Neurofibromatosis Foundation International Database. Am J Med Genet 45:88–91.

Habiby R, Silverman B, Listernick R, Charrow J (1995): Precocious puberty in children with neurofibromatosis type 1. J Pediatr 126:364–367.

Hoekelman RA, Blatman S, Friedman SB, Nelson NM, Seidel HM (eds) (1987): "Primary Pediatric Care." St. Louis: C.V. Mosby Co., pp 1469.

Hughes JG, Griffith JF (1984): "Synopsis of Pediatrics," 6th Ed. St. Louis: C.V. Mosby Co.

Huson SM (1994): Neurofibromatosis 1: A clinical and genetic overview. In Huson SM, Hughes RAC (eds): "The Neurofibromatoses: A Pathogenetic and Clinical Overview." London: Chapman and Hall Medical, pp 160–204.

Huson SM, Compston DAS, Clark P, Harper PS (1989): A genetic study of von Recklinghausen neurofibromatosis in south east Wales. Prevalence, fitness, mutation rate, and effect of parental transmission on severity. J Med Genet 26:704–711.

Mulvihill JJ (1990): Introduction and History. In Rubenstein A, Korf B (eds): "Neurofibromatosis: A Handbook for Patients, Families and Health Care Professionals." New York: Thieme, pp 1–12.

NIH Consensus Development Conference: Neurofibromatosis (1988): Arch Neurol 45:575–578.

North K (1993): Neurofibromatosis Type 1: Review of the first 200 patients in an Australian clinic. J Child Neurol 8:395–402.

Riccardi VM (1992): "Neurofibromatosis: Phenotype, Natural History and Pathogenesis," 2nd Ed. Baltimore: Johns Hopkins University Press.

Rubenstein A, Korf B (eds) (1990): "Neurofibromatosis: A Handbook for Patients, Families and Health Care Professionals." New York: Thieme.

Samuelsson B, Axelsson R (1981): Neurofibromatosis: A clinical and genetic study of 96 cases in Gothenburg, Sweden. Acta Dermatovenereologica Suppl 95:67–71.

Systat Inc. (1990): "Systat 5.0." Evanston, Illinois.

Zar JH (1984): "Biostatistical Analysis," 2nd Ed. London: Prentice Hall, pp 48.

Zvulunov A, Barak Y, Metzker A (1995): Juvenile xanthogranuloma, neurofibromatosis, and juvenile chronic myelogenous leukemia. World statistical analysis. Arch Dermatol 131:904–908.

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ARTICLES

Epidemiology of Neurofibromatosis Type 1

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The prevalence of neurofibromatosis type 1 (NF1) is about 1/3,000. There are no known ethnic groups in which NF1 does not occur or is unusually common. The prevalence is somewhat higher in young children than in adults, a difference that probably results at least in part from the early death of some NF1 patients. NF1 is fully penetrant in adults, but many disease features increase in frequency or severity with age. The reproductive fitness of NF1 patients is reduced by about one-half. About half of all cases result from new mutations. The estimated rate of new *NF1* mutations is unusually high, but the basis for this high mutation rate is not known. Am. J. Med. Genet. (Semin. Med. Genet.) 89:1–6, 1999. © 1999 Wiley-Liss, Inc.

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INTRODUCTION

Neurofibromatosis type 1 (NF1) is one of the most common Mendelian diseases, but its natural history and genetic epidemiology are incompletely understood. NF1 is characterized by autosomal dominant inheritance with complete penetrance but extremely variable expression. The life expectancy and reproductive fitness of patients with NF1 are lower than normal, but the causes have not been clearly defined. The source of the remarkably high rate of new mutations at the *NF1* locus is also unknown. This article reviews current knowledge of the prevalence, fitness, and disease burden of NF1 and of the frequency, occurrence, and penetrance of associated *NF1* mutations.

PREVALENCE

Estimates of the prevalence of NF1 range from 1/2,190 to 1/7,800. The studies upon which these estimates are based are summarized in Table 1. The lowest reported prevalence is from a study of healthy army recruits [Sergeyev, 1975], a group that might be expected to exclude all severely affected NF1 patients. Some NF1 patients were probably missed in the other populations studied because mildly affected individuals do not come to medical attention [Huson et al., 1989a; Clementi et al., 1990]. On the other hand, a few patients who did not actually have NF1 may have been counted as affected in some of the earlier studies. Only the study of Poyhonen et al. [1997] used the full NIH diagnostic criteria to identify NF1 patients [National Institutes of Health Consensus Development Conference, 1988; Gutmann et al., 1997]. The prevalence of NF1 was estimated to be about 1/3,700 in this study, but ascertainment was probably incomplete because it was based on hospital records.

An important factor in interpreting these investigations is the age of the population studied. Clementi et al. [1990] observed a higher prevalence of NF1 among children under 9 years of age, a group that was excluded from the studies of Sergeyev [1975] and Samuelsson and colleagues [Samuelsson and Axelsson, 1981; Samuelsson and Samuelsson, 1989]. Huson and associates [1988, 1989a] also found a higher prevalence of NF1 among younger individuals. Correcting for less than complete ascertainment, Huson et al. [1989a] estimated the incidence of *NF1* mutation carriers at birth to be 1/2,558. The finding of a higher prevalence of NF1 among younger people may result from earlier death among NF1 patients [Huson et al., 1988; Fuller et al., 1989; Samuelsson and Samuelsson, 1989]. It is not clear, however, that this effect is severe enough to explain the magnitude of the observed difference in NF1 prevalence between children and adults. Underascertainment of adult patients may also contribute to the higher observed prevalence of NF1 among children [Clementi et al., 1990].

The prevalence of NF1 has been measured only in Caucasian and Japanese populations, but there is no evidence that ethnic differences greatly affect the prevalence. Patients and families with NF1 have been reported from throughout the world. There is no population in which NF1 is known not to occur, and most serious complications are observed with similar frequencies in different ethnic groups [Huson et al., 1989b; Niimura, 1990; Riccardi, 1992; Wong, 1994; Friedman and Birch, 1997a; Poyhonen et al., 1997;

The prevalence of NF1 has been measured only in Caucasian and Japanese populations, but there is no evidence that ethnic differences affect the frequency greatly.

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TABLE I. Estimates of the Prevalence of NF1

Study	Population studied	Estimated prevalence
Borberg, 1951; Littler and Morton, 1990	Hospitalized patients in Denmark, 1924–1944	1/3,704
Crowe et al., 1956	Patients admitted to University of Michigan Hospital, 1934–1950 or 1951–1953, or resident in a state institution for the mentally retarded or epileptic	1/2,500–1/3,300
Sergeyev, 1975	16-year old Russian male army recruits	1/7,800
Samuelsson and Axelsson, 1981; Samuelsson and Samuelsson, 1989	Patients 20 years of age or older known to the health services in Gothenburg, Sweden, on January 1, 1978	1/4,600
Huson, et al., 1988, 1989a	Patients in south Glamorgan and west Gwent, Wales, identified through hospital records (1954–, 1969–, or 1972–1986), physician contacts, and public appeal	1/4,150–1/4,950 (1/2,558 at birth)
Fuller et al., 1989	Patients in greater Dunedin, New Zealand, identified through hospital and medical genetics records (1961–1988) and physician contacts	1/2,190
Clementi et al., 1990	Patients referred for genetic counseling from Veneto or Friuli-Venzia Giulia, Italy	1/6,711
Poyhonen et al., 1997	Patients identified through hospital records in northern Finland	1/3,716

Cnossen et al., 1998]. One exception may be optic glioma, which appears to be less common among black than white NF1 patients. In contrast, carcinoid tumours may be more likely to develop in black NF1 patients [Burke et al., 1990].

PENETRANCE

The penetrance of pathogenic *NF1* mutations in adults is complete or very nearly so. No cases of nonpenetrance were observed in thorough clinical studies of some 200 multigeneration families [Crowe et al., 1956; Sergeyev, 1975; Riccardi and Lewis, 1988; Huson et al., 1989a; Samuelsson and Akesson, 1989; Riccardi 1992]. Although anecdotal instances of nonpenetrance have been reported [Carey et al., 1979; Spence et al., 1983], no case has been documented with mutation analysis or linkage and full clinical evaluation using the NIH diagnostic criteria.

Although NF1 exhibits virtually complete penetrance in adults, most manifestations of the disease increase in frequency with age among affected children [Huson et al., 1989b; Obringer et al., 1989; Niimura, 1990; Huson, 1994; Wolkenstein et al., 1996; Friedman and Birch, 1997a; DeBella et al., 1999]. NF1 cannot be diagnosed using the NIH criteria in many infants and young children who carry *NF1* mutations [Riccardi, 1981; Huson et al., 1989b; Orbringer et al., 1989; Korf, 1992; Goldberg et al., 1996]. The disease is apparent, however, in almost all affected individuals by 8 years of age and in 100% by age 20 [Obringer et al., 1989; DeBella et al., 1999].

GENE FREQUENCY

NF1 is an autosomal dominant disease. There is no evidence of locus heterogeneity; that is, all patients with typical NF1 appear to have mutations at the same locus [Collins et al., 1989; Ward et al., 1990; Riccardi, 1993; Marchuk and Collins, 1994]. Individuals with NF1 are heterozygotes for an *NF1* mutation. No convincing example of homozygous NF1 has been reported, despite the high disease prevalence and known instances of consanguinity in families in which NF1 segregates [Pericak-Vance et al., 1987; Vance et al., 1989]. The fact that homozygosity for mutations of the *NF1* homologue is lethal in mouse embryos [Jacks et al., 1994; Brannan et al., 1994] suggests that at least one functional *NF1* allele is essential in early fetal development. Given that NF1 is an autosomal dominant disease with virtually complete penetrance and no locus heterogeneity, the frequency of mutant *NF1* genes can be estimated simply as half the incidence of the disease at birth. Estimates of disease prevalence range from 1/2,190 to 1/7,800, with the higher values more closely approximating birth incidence (see earlier). Therefore, the mutant gene frequency is probably about 1/5,000 (0.0002).

NEW MUTATIONS

A positive family history is present in about half of all NF1 cases [Crowe et al., 1956; Brasfield and Das Gupta, 1972; Carey et al., 1979; Huson et al., 1989a; Samuelsson and Akesson, 1989; Clementi et al., 1990; Littler and Morton, 1990; Riccardi, 1992; Takano et al., 1992; North, 1993; Poyhonen et al., 1997]. If one assumes that penetrance is complete, this means that about half of all NF1 patients represent new mutations. Estimates of the rate of new *NF1* mutations vary from 1/7,800 to 1/23,000 gametes [Crowe et al., 1956; Sergeyev, 1975; Samuelsson and Akesson, 1989; Huson et al., 1989a; Clementi et al., 1990; Littler and Morton, 1990; Takano et al., 1992]. This

range reflects differences in diagnosis and ascertainment of sporadic cases as well as statistical fluctuations and differences in the method used to calculate the mutation rate. Nevertheless, it is clear that NF1 has one of the highest single locus mutation rates known in humans.

The reasons for this high mutation rate are not understood. Several explanations have been proposed, and more than one may play a part. Most *NF1* mutations are unique to a single family. The large size of the *NF1* gene may be an important factor, but this alone does not appear to be a sufficient explanation [Upadyaya et al., 1994; Rodenhiser et al., 1997]. Palindromes, symmetrical elements, and runs of repeated sequences have been associated with preferential occurrence of insertions and deletions in some genes [Cooper and Krawczak, 1991; Krawczak and Cooper, 1991; Rodenhiser et al., 1997]. Many *NF1* deletions and insertions occur within such sequences, but many other *NF1* mutations are substitutions rather than insertions or deletions [Shen et al., 1996; Rodenhiser et al., 1997].

Pathogenetic substitutions in the *NF1* gene are often found in homonucleotide repeats or CpG dinucleotides [Rodenhiser et al., 1997]. Methylated CpG dinucleotides often show a high mutation rate because of the tendency of 5-methylcytosine to undergo spontaneous deamination to thiamine [Coulondre et al., 1978]. The most commonly reported *NF1* mutation is a transition that involves a single CpG site in exon 31 [Shen et al., 1996]. However, only a small fraction of *NF1* mutations occur at CpG sites [Shen et al., 1996; Rodenhiser et al., 1997].

Genes that show substantial sequence similarity to *NF1* exist on chromosomes 2, 12, 14, 15, 18, 20, 21, and 22 [Legius et al., 1992; Gasparini et al., 1993; Purandare et al., 1995; Ritchie et al., 1998]. Gene conversion between a non-homologous pseudogene and *NF1* might produce some mutations [Upadhyaya et al., 1994; Purandare et al., 1995; Shen et al., 1996], but direct sequence comparisons between mutations and known pseudogenes did not show evidence of gene conversion events in one study [Purandare et al., 1995].

It is possible that an *NF1* mutation in a germ-cell precursor provides a proliferative advantage to that cell and its progeny [Rodenhiser et al., 1997]. Such an effect would produce germline mosaicism that increases the likelihood of a given mutation being transmitted to a child. This would elevate the apparent (but not actual) mutation rate for *NF1*. Germ-line mosaicism has been established in one individual with no clinical features of NF1 but who had two affected children [Lázaro et al., 1994, 1995]. Germ-line mosaicism is also thought to explain the fact that several other families have more than one child with NF1 born to unaffected parents [Berry et al., 1984; Riccardi and Lewis, 1988].

More than 80% of new NF1 mutations are of paternal origin . . . If a paternal age effect exists for NF1 mutations, it is modest.

PARENT OF ORIGIN OF NEW MUTATIONS

More than 80% of new *NF1* mutations are of paternal origin [Jadayel et al., 1990; Spiegel et al., 1991; Stephens et al., 1992; Elyakim et al., 1994; Upadhyaya et al., 1994; Lázaro et al., 1996]. "Large" submicroscopic deletions of all or most of the *NF1* gene, which account for no more than 5% of all *NF1* mutations, are an exception to this rule. Most such deletions are of maternal rather than paternal origin [Lázaro et al., 1996; Ainsworth et al., 1997; Valero et al., 1997; Upadhyaya et al., 1998]. A predominance of paternally derived germ-line mutations has been observed in the context of several other genetic diseases that result from diverse mutational mechanisms [Kling et al., 1992; Goldberg et al., 1993; Palau et al., 1993; Carlson et al., 1994; Kato et al., 1994; Moloney et al., 1996; Dryja et al., 1997; Wirth et al., 1997]. The predominance of paternal mutations in *NF1* is, therefore, unlikely to provide an important clue to the cause of the high mutation rate.

Given the predominance of paternal mutations at the *NF1* locus, one might expect the risk of having a child with sporadic NF1 to increase with paternal age. Available data regarding paternal age in NF1 are inconsistent [Borberg, 1951; Sergeyev, 1975; Riccardi et al., 1984; Huson et al., 1989a; Samuelsson and Akesson, 1989; Clementi et al., 1990; Takano et al., 1992; North, 1993; Bunin et al., 1997]. The largest reported study of this issue is that of Riccardi et al. [1984], which included 187 NF1 patients born to apparently unaffected parents. A small, but statistically significant, increase in the age of the fathers of the patients was observed when compared with the expected paternal age calculated from national averages and weighted by each proband's year of birth. A statistically significant effect was also seen using other methods of analysis that control for maternal age. Unfortunately, this and all other available investigations of the paternal age effect are compromised by uncertainty about the comparability of case and control groups. Incomplete ascertainment of NF1 cases, lack of unequivocal demonstration that all cases actually represent new mutations, and small sample size are also problems in some studies. The only conclusion that can be made on the basis of currently available data is that if a paternal age effect exists for *NF1* mutations, it is modest.

FITNESS

Crowe et al. [1956] estimated the fitness of NF1 patients to be 0.53, that is, about half of normal. Similar figures (0.47 and 0.54, respectively) were obtained by Huson et al. [1989a] and by Takano et al. [1992]. Both Crowe and associates and Huson and colleagues found the fitness to be much lower for affected men than for affected women.

Reproductive fitness is a function of all factors that affect a person's ability to have children. Reduced fitness means that people with NF1 have fewer

children, on average, than other people. Diminished reproductive fitness could occur if people who carry a pathogenetic *NF1* mutation were more often infertile or subfertile, if NF1 embryos were more often miscarried, if stillbirth were more common in NF1 fetuses, if children with NF1 more often died before reaching reproductive maturity, or if adults with NF1 were less likely to have children for medical or social reasons. These possibilities are not mutually exclusive, and several of them may play a role in the reduced reproductive fitness of NF1 patients.

The average life-span of people with NF1 is reduced, but most of this excess mortality occurs after the age at which people usually have children.

Only limited data are available to assess these factors. Several studies have found that 50% of children of an affected parent have NF1 [Crowe et al., 1956; Brasfield and Das Gupta, 1972; Sergeyev, 1975; Huson et al., 1989a; Samuelsson and Akesson, 1989; Clementi et al., 1990; Littler and Morton, 1990; Riccardi, 1992; North, 1993]. This result suggests that prenatal loss of fetuses that carry an *NF1* mutation is not excessive. Riccardi and Eichner [1986] found that the mean number of offspring of NF1 patients who had children did not differ significantly from the mean number of children of unaffected sibs, but this analysis was based on a small sample. Crowe and associates [1956] attributed about half of the reduced fitness among NF1 patients to lower rates of marriage, an effect that was more marked in men than in women.

MORBIDITY AND MORTALITY

Another possible cause of diminished reproductive fitness is the increased morbidity and mortality associated with NF1. NF1 is a progressive, although unpredictable, disease associated with a variety of clinical manifestations and complications [Sørensen et al., 1986a; Huson et al., 1989a, 1989b; Samuelsson and Samuelsson, 1989; Riccardi, 1992; Zöller et al., 1995; Friedman and Birch, 1997a; Poyhonen et al., 1997]. The average life span of people with NF1 is lower, but most of deaths occur after the age at which people usually have children.

Two population-based long-term follow-up studies provide the best available mortality data on NF1 patients. Sørensen and associates [1986b] estimated survival over 39 years for 76 NF1 probands and 79 of their affected relatives identified through Danish hospital records [Borberg, 1951]. Survival was significantly shortened among the NF1 patients compared with the general population. The most common causes of death—cancer, myocardial infarction, cerebrovascular accidents, and pneumonia—were similar to those in the general population but often occurred earlier in life. Central nervous system tumours, especially gliomas, and second primary neoplasms appeared to be unusually common among NF1 patients.

The series reported by Zöller and colleagues [1995] includes information on 70 Swedish NF1 patients whose average age was 43.6 years at the time of ascertainment. Over the next 12 years, 22 of these patients died, whereas 5.1 deaths would have been expected in the general population. The mean age at death for the NF1 patients was 61.6 years. The average life expectancy for the Swedish population from which these patients were drawn was 75 years. Malignancy, the most common cause of death, occurred in 17 (24%) of the 70 patients reported by Zöller et al. [1995, 1997]. Hypertension was significantly associated with mortality–10 of 12 NF1 patients with high blood pressure died during the period of observation. Cardiovascular disease, hemorrhage, or embolism caused the death of seven people, but these deaths may have been unrelated to NF1 in some or all cases.

NF DATABASES

Several electronic databases have been established to facilitate neurofibromatosis research. The National Neurofibromatosis Foundation (NNFF) International Database contains extensive demographic, clinical, and genetic data on more than 3,000 NF1 patients, including about 400 multiplex families. This information has been collected from 26 participating centers in North America, Europe, Japan, and Australia. The database is maintained by Dr. J. M. Friedman and Patricia Birch at the University of British Columbia, to encourage comprehensive clinical and genetic analysis of neurofibromatosis [Friedman et al., 1993]. All data are recorded using a standard format and consistent definitions of clinical features.

The greatest strength of the NNFF International Database is its large size, which has permitted use for such purposes as developing standard growth charts for children with NF1 [Szudek et al., 1998], estimation of the frequencies of various disease manifestations by age [Friedman and Birch, 1997a; DeBella et al., 1999], and demonstration of associations between the occurrence of clinical features in NF1 probands and family members [Friedman and Birch, 1997b; Szudek et al., 1997]. Limitations include the fact that most contributors are NF clinics at major medical centres, which probably produces an ascertainment bias. Data quality is somewhat variable because the NNFF International Database depends entirely on voluntary contributions from clinicians throughout the world.

The Neurofibromatosis Institute Database contains information on the large series of patients that Dr. V. M. Riccardi has evaluated personally. The NF Institute Database includes standard clinical information on more than 1,100 NF1 patients. Longitudinal data are available over several years on about one-third of these patients. These data have been described in detail [Riccardi and Eichner, 1986; Riccardi, 1992]. The information in the Neurofibromatosis Institute Database is particularly valuable because of its consistency. An enormous amount of information is available on each patient; in many instances this includes results of laboratory and radiological studies as well very detailed clinical descriptions. One limita-

tion is that most of these data were collected before the NIH diagnostic criteria were established, and some variables were recorded in a format that does not permit direct assessment of these criteria.

Qualified investigators can request information from either of these electronic databases. Their use can help address some of the unanswered questions about the genetic epidemiology of NF1. Population-based studies, long-term longitudinal studies, detailed family studies, and investigations of genotype–phenotype correlations are also necessary. Better understanding of the natural history, variability, and causes of the increased mortality of NF1 are essential to improving the lives of people with this common genetic disease.

REFERENCES

Ainsworth PJ, Chakraborty PK, Weksberg R. 1997. Example of somatic mosaicism in a series of de novo neurofibromatosis type 1 cases due to a maternally derived deletion. Hum Mutat 9:452–457.

Berry SA, King RA, Whitley CB, Riccardi VM, Pierpont MEM. 1984. Familial neurofibromatosis resulting from a probable germinal mutation. Am J Hum Genet 36:44s.

Borberg A. 1951. Clinical and genetic investigations into tuberous sclerosis and Recklinghausen's neurofibromatosis: contributions to elucidation of interrelationship and eugenics of the syndromes. Acta Psychiatr Neurol Scand 71(suppl):1–239.

Brannan CI, Perkins AS, Vogel KS, Ratner N, Norlund ML, Reid SW, Buchberg AM, Jenkins NA, Parada LF, Copeland NG. 1994. Targeted disruption of the neurofibromatosis type-1 gene leads to developmental abnormalities in heart and various neural crest–derived tissues. Genes Dev 8: 1019–1029.

Brasfield RD, Das Gupta TK. 1972. Von Recklinghausen's disease: a clinicopathological study. Ann Surg 175:86–104.

Bunin GR, Needle M, Riccardi VM. 1997. Paternal age and sporadic neurofibromatosis 1: a case-control study and consideration of methodologic issues. Genet Epidemiol 14: 507–516.

Burke AP, Sobin LH, Shekitka KM, Federspiel BH, Helwig EB. 1990. Somatostatin-producing duodenal carcinoid in patients with von Recklinghausen's neurofibromatosis: a predilection for black patients. Cancer 65:1591–1595.

Carey JC, Laub JM, Hall BD. 1979. Penetrance and variability in neurofibromatosis: a genetic study of 60 families. Birth Defects 15(5B):271–281.

Carlson KM, Bracamontes J, Jackson CE, Clark R, Lacroix A, Wells SA, Goodfellow PJ. 1994. Parent-of-origin effects in multiple endocrine neoplasia type 2B. Am J Hum Genet 55:1076–1082.

Clementi M, Barbujani G, Turolla L, Tenconi R. 1990. Neurofibromatosis-1: a maximum likelihood estimation of mutation rate. Hum Genet 84:116–118.

Cnossen MH, de Goede-Bolder A, van den Broek KM, Waasdorp CME, Oranje AP, Stroink H, Simonsz HJ, van den Ouweland AMW, Halley DJJ, Niermeijer MF. 1998. A prospective 10 year follow up study of patients with neurofibromatosis type 1. Arch Dis Child 78:408–412.

Collins FS, O'Connell P, Ponder BAJ, Seizinger BR. 1989. Progress towards identifying the neurofibromatosis (NF1) gene. Trends Genet 5:217–221.

Cooper DN, Krawczak M. 1991. Mechanisms of insertional mutagenesis in human genes causing genetic disease. Hum Genet 87: 409–415.

Coulondre C, Miller JH, Farabaugh PJ, Gilbert W. 1978. Molecular basis of base substitution hotspots in *Escherichia coli*. Nature 274: 775–780.

Crowe FW, Schull WJ, Neel JV. 1956. A clinical, pathological, and genetic study of multiple neurofibromatosis. Springfield, Ill: Charles C Thomas.

DeBella K, Szudek J, Friedman JM. 1999. Use of the NIH criteria for diagnosis of NF1 in children. Pediatrics (in press).

Dryja TP, Morrow JF, Rapaport JM. 1997. Quantification of the paternal allele bias for new germline mutations in the retinoblastoma gene. Hum Genet 100:446–449.

Elyakim S, Lerer I, Zlotogora J, Sagi M, Gelman-Kohan Z, Merin S, Abeliovich D. 1994. Neurofibromatosis type I (NFI) in Israeli families: linkage analysis as a diagnostic tool. Am J Med Genet 53:325–334.

Friedman JM, Birch PH. 1997a. Type 1 neurofibromatosis: a descriptive analysis of the disorder in 1,728 patients. Am J Med Genet 70:138–143.

Friedman JM, Birch P. 1997b. An association between optic glioma and other tumours of the central nervous system in neurofibromatosis type 1. Neuropediatrics 28:131–132.

Friedman JM, Green C, Birch P, and the NNFF International Database Participants. 1993. National Neurofibromatosis Foundation International Database. Am J Med Genet 45:88–91.

Fuller LC, Cox B, Gardner RJM. 1989. Prevalence of von Recklinghausen neurofibromatosis in Dunedin, New Zealand. Neurofibromatosis 2:278–283.

Gasparini P, Grifa A, Origone P, Coviello D, Antonacci R, Rocchi M. 1993. Detection of a neurofibromatosis type I (NF1) homologous sequence by PCR: implications for the diagnosis and screening of genetic diseases. Mol Cell Probes 7:415–418.

Goldberg Y, Dibbern K, Klein J, Riccardi VM, Graham JM. 1996. Neurofibromatosis type 1: an update and review for the primary pediatrician. Clin Pediatr (Phila) 35:545–561.

Goldberg YP, Kremer B, Andrew SE, Theilmann J, Graham RK, Squitieri F, Telenius H, Adam S, Sajoo A, Starr E, Heiberg A, Wolff G, Hayden MR. 1993. Molecular analysis of new mutations for Huntington's disease: intermediate alleles and sex of origin effects. Nature Genet 5:174–179.

Gutmann DH, Aylsworth A, Carey JC, Korf B, Marks J, Pyeritz RE, Rubenstein A, Viskochil D. 1997. The diagnostic evaluation and multidisciplinary management of neurofibromatosis 1 and neurofibromatosis 2. JAMA 278:51–57.

Huson SM. 1994. Neurofibromatosis 1: a clinical and genetic overview. In: Huson SM, Hughes RAC, editors. The neurofibromatoses: a pathogenic and clinical overview. London: Chapman & Hall. p 160–203.

Huson SM, Compston DAS, Clark P, Harper PS. 1989a. A genetic study of von Recklinghausen neurofibromatosis in southeast Wales. I. Prevalence, fitness, mutation rate, and effect of parental transmission on severity. J Med Genet 26:704–711.

Huson SM, Compston DAS, Harper PS. 1989b. A genetic study of von Recklinghausen neurofibromatosis in southeast Wales. II. Guidelines for genetic counselling. J Med Genet 26:712–721.

Huson SM, Harper PS, Compston DAS. 1988. Von Recklinghausen neurofibromatosis: a clinical and population study in South-East Wales. Brain 111:1355–1381.

Jacks T, Shih TS, Schmitt EM, Bronson RT, Bernards A, Weinberg RA. 1994. Tumour predisposition in mice heterozygous for targeted mutation of *Nf1*. Nature Genet 7: 353–361.

Jadayel D, Fain P, Upadhyaya M, Ponder MA, Huson SM, Carey J, Fryer A, Mathew CGP, Barker DF, Ponder BAJ. 1990. Paternal origin of new mutations in von Recklinghausen neurofibromatosis. Nature 343:558–559.

Kato MV, Ishizaki K, Shimizu T, Ejima Y, Tanooka H, Takayama J, Kaneko A, Toguchida J, Sasaki MS. 1994. Parental origin of germ-line and somatic mutations in the retinoblastoma gene. Hum Genet 94:31–38.

Kling S, Ljung R, Sjörin E, Montandon J, Green P, Giannelli F, Nilsson IM. 1992. Origin of mutation in sporadic cases of haemophilia-B. Eur J Haematol 48:142–145.

Korf BR. 1992. Diagnostic outcome in children with multiple café au lait spots. Pediatrics 90:924–927.

Krawczak M, Cooper DN. 1991. Gene deletions causing human genetic disease: mechanisms of mutagenesis and the role of the local DNA sequence environment. Hum Genet 86:425–444.

Lázaro C, Gaona A, Ainsworth P, Tenconi R, Vidaud D, Kruyer H, Ars E, Volpini V, Estivill X. 1996. Sex differences in mutational rate and mutational mechanism in the *NF1* gene in neurofibromatosis type 1 patients. Hum Genet 98:696–699.

Lázaro C, Gaona A, Lynch M, Kruyer H, Ravella A, Estivill X. 1995. Molecular characterization of the breakpoints of a 12-kb deletion in the *NF1* gene in a family showing germline mosaicism. Am J Hum Genet 57:1044–1049.

Lázaro C, Ravella A, Gaona A, Volpini V, Estivill X. 1994. Neurofibromatosis type 1 due to germ-line mosaicism in a clinically normal father. N Engl J Med 331:1403–1407.

Legius E, Marchuk DA, Hall BK, Andersen LB, Wallace MR, Collins FS, Glover TW.

1992. *NF-1* related locus on chromosome 15. Genomics 13:1316–1318.

Littler M, Morton NE. 1990. Segregation analysis of peripheral neurofibromatosis (NF1). J Med Genet 27:307–310.

Marchuk DA, Collins FS. 1994. Molecular genetics of neurofibromatosis 1. In: Huson SM, Hughes RAC, editors. The neurofibromatoses: a pathogenic and clinical overview. London: Chapman & Hall. p 22–49.

Moloney DM, Slaney SF, Oldridge M, Wall SA, Sahlin P, Stenman G, Wilkie AOM. 1996. Exclusive paternal origin of new mutations in Apert syndrome. Nature Genet 13:48–53.

National Institutes of Health Consensus Development Conference. 1988. Neurofibromatosis: Conference statement. Arch Neurol 45:575–578.

Niimura M. 1990. Neurofibromatosis in Japan. In: Ishibashi Y, Hori Y, editors. Tuberous sclerosis and neurofibromatosis: epidemiology, pathophysiology, biology, and management. Amsterdam: Elsevier. p 23–31.

North K. 1993. Neurofibromatosis type 1: review of the first 200 patients in an Australian clinic. J Child Neurol 8:395–402.

Obringer AC, Meadows AT, Zackai EH. 1989. The diagnosis of neurofibromatosis-1 in the child under the age of 6 years. Am J Dis Child 143:717–719.

Palau F, Löfgren A, De Jonghe P, Bort S, Nelis E, Sevilla T, Martin J-J, Vilchez J, Prieto F, van Broeckhoven C. 1993. Origin of the *de novo* duplication in Charcot-Marie-Tooth disease type 1A: unequal non-sister chromatid exchange during spermatogenesis. Hum Mol Genet 2:2031–2035.

Pericak-Vance MA, Yamaoka LH, Vance JM, Small K, Rosenwasser GOD, Gaskell PC, Hung W-Y, Alberts MJ, Haynes CS, Speer MC, Gilbert JR, Herbstreith M, Aylsworth AS, Roses AD. 1987. Genetic linkage studies on chromosome 17 RFLPs in von Recklinghausen neurofibromatosis (NF-1). Genomics 1:349–352.

Poyhonen M, Niemela S, Herva R. 1997. Risk of malignancy and death in neurofibromatosis. Arch Pathol Lab Med 121:139–143.

Purandare SM, Breidenbach HH, Li Y, Zhu XL, Sawada S, Neil SM, Brothman A, White R, Cawthon R, Viskochil D. 1995. Identification of neurofibromatosis 1 (*NF1*) homologous loci by direct sequencing, fluorescence *in situ* hybridization, and PCR amplification of somatic cell hybrids. Genomics 30:476–485.

Riccardi VM. 1981. Von Recklinghausen neurofibromatosis. N Engl J Med 305:1617–1627.

Riccardi VM. 1992. Neurofibromatosis: phenotype, natural history, and pathogenesis, 2nd ed. Baltimore: Johns Hopkins University Press.

Riccardi VM, 1993. Genotype, malleotype, phenotype, and randomness: lessons from neurofibromatosis-I (NF-I). Am J Hum Genet 53:301–304.

Riccardi VM, Dobson CE, Chakraborty R, Bontke C. 1984. The pathophysiology of neurofibromatosis: IX. Paternal age as a factor in the origin of new mutations. Am J Med Genet 18:169–176.

Riccardi VM, Eichner JE. 1986. Neurofibromatosis: phenotype, natural history, and pathogenesis. Baltimore: Johns Hopkins University Press.

Riccardi VM, Lewis RA. 1988. Penetrance of von Recklinghausen neurofibromatosis: a distinction between predecessors and descendants. Am J Hum Genet 42:284–289.

Ritchie RJ, Mattei MG, Lalande M. 1998. A large polymorphic repeat in the pericentromeric region of human chromosome 15q contains three partial gene duplications. Hum Mol Genet 7:1253–1260.

Rodenhiser DI, Andrews JD, Mancini DN, Jung JH, Singh SM. 1997. Homonucleotide tracts, short repeats and CpG/CpNpG motifs are frequent sites for heterogeneous mutations in the neurofibromatosis type 1 (NF1) tumour-suppressor gene. Mutat Res 373:185–195.

Samuelsson B, Akesson HO. 1989. Neurofibromatosis in Gothenburg, Sweden. IV. Genetic analyses. Neurofibromatosis 2:107–115.

Samuelsson B, Axelsson R. 1981. Neurofibromatosis: a clinical and genetic study of 96 cases in Gothenburg, Sweden. Acta Derm Venereol Suppl (Stockh) 95:67–71.

Samuelsson B, Samuelsson S. 1989. Neurofibromatosis in Gothenburg, Sweden. I. Background, study design and epidemiology. Neurofibromatosis 2:6–22.

Sergeyev AS. 1975. On the mutation rate of neurofibromatosis. Hum Genet 28:129–138.

Shen MH, Harper PS, Upadhyaya M. 1996. Molecular genetics of neurofibromatosis type 1 (NF1). J Med Genet 33:2–17.

Sørensen SA, Mulvihill JJ, Nielsen A. 1986a. On the natural history of von Recklinghausen neurofibromatosis. Ann N Y Acad Sci 486: 30–37.

Sørensen SA, Mulvihill JJ, Nielsen A. 1986b. Long-term follow-up of von Recklinghausen neurofibromatosis: survival and malignant neoplasms. N Engl J Med 314:1010–1015.

Spence MA, Bader JL, Parry DM, Field LL, Funderburk SJ, Rubenstein AE, Gilman PA, Sparkes RS. 1983. Linkage analysis of neurofibromatosis (von Recklinghausen disease). J Med Genet 20:334–337.

Spiegel R, Mächler M, Stocker HP, Boltshauser E, Schmid W. 1991. Neurofibromatose Typ 1: genetische Untersuchungen mit DNA-Markern bei 38 Familien. Schweiz Med Wschr 121:1445–1452.

Stephens K, Kayes L, Riccardi VM, Rising M, Sybert VP, Pagon RA. 1992. Preferential mutation of the neurofibromatosis type 1 gene in paternally derived chromosomes. Hum Genet 88:279–282.

Szudek J, Birch P, Friedman JM, the National Neurofibromatosis Foundation International Database Participants. 1998. Height and head circumference in patients with neurofibromatosis type 1 (NF1). Am J Hum Genet 63:A122.

Szudek J, Riccardi VM, Friedman JM. 1997. Associations of clinical features in children with neurofibromatosis type 1 (NF1). Am J Hum Genet 61:A115.

Takano T, Kawashima T, Yamanouchi Y, Kitayama K, Baba T, Ueno K, Hamaguchi H. 1992. Genetics of neurofibromatosis 1 in Japan: mutation rate and paternal age effect. Hum Genet 89:281–286.

Upadhyaya M, Ruggieri M, Maynard J, Osborn M, Hartog C, Mudd S, Penttinen M, Cordeiro I, Ponder M, Ponder BAJ, Krawczak M, Cooper DN. 1998. Gross deletions of the neurofibromatosis type 1 (*NF1*) gene are predominantly of maternal origin and commonly associated with a learning disability, dysmorphic features and developmental delay. Hum Genet 102:591–597.

Upadhyaya M, Shaw DJ, Harper PS. 1994. Molecular basis of neurofibromatosis type 1 (NF1): mutation analysis and polymorphisms in the NF1 gene. Hum Mutat 4:83–101.

Valero MC, Pascual-Castroviejo I, Velasco E, Moreno F, Hernádez-Chico C. 1997. Identification of de novo deletions at the NF1 gene: no preferential paternal origin and phenotypic analysis of patients. Hum Genet 99:720–726.

Vance JM, Pericak-Vance MA, Yamaoka LH, Speer MC, Rosenwasser GOD, Small K, Gaskell PC, Hung W-Y, Alberts MJ, Haynes CS, Gilbert JR, Aylsworth AS, Roses AD. 1989. Genetic linkage mapping of chromosome 17 markers and neurofibromatosis type 1. Am J Hum Genet 44:25–29.

Ward K, O'Connell PO, Carey JC, Leppert M, Jolley S, Plaetke R, Ogden B, White R. 1990. Diagnosis of neurofibromatosis I by using tightly-linked flanking DNA markers. Am J Hum Genet 46:943–949.

Wirth B, Schmidt T, Hahnen E, Rudnik-Schöneborn S, Krawczak M, Müller-Myhsok B, Schönling J, Zerres K. 1997. De novo rearrangements found in 2% of index patients with spinal muscular atrophy: mutational mechanisms, parental origin, mutation rate, and implications for genetic counseling. Am J Hum Genet 61:1102–1111.

Wolkenstein P, Frèche B, Zeller J, Revuz J. 1996. Usefulness of screening investigations in neurofibromatosis type 1: a study of 152 patients. Arch Dermatol 132:1333–1336.

Wong V C-N. 1994. Clinical manifestations of neurofibromatosis-1 in Chinese children. Pediatr Neurol 11:301–307.

Zöller M, Rembeck B, Åkesson HO, Angervall L. 1995. Life expectancy, mortality and prognostic factors in neurofibromatosis type 1: a twelve-year follow-up of an epidemiological study in Göteborg, Sweden. Acta Derm Venereol (Stockh) 75:136–140.

Zöller M, Rembeck B, Odén A, Samuelsson M, Agervall L. 1997. Malignant and benign tumors in patients with neurofibromatosis type 1 in a defined Swedish population. Cancer 79:2125–2131.

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Mini Review

Insights into the pathogenesis of neurofibromatosis 1 vasculopathy

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Intrinsic lesions of arterial walls are an important manifestation of neurofibromatosis 1 (NF1). Neurofibromin is expressed in blood vessel endothelial and smooth muscle cells, and NF1 vasculopathy may result from an alteration of neurofibromin function in these cells. Elucidation of the role of neurofibromin in the maintenance and repair of blood vessels may lead to novel approaches to the treatment of NF1 vasculopathy and vascular disease in general.

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Neurofibromatosis 1 (NF1) is among the most common inherited human diseases (1). Its most frequent manifestations include café-au-lait spots and dermal neurofibromas, which result from abnormalities in neural crest-derived cell types. Many non-neural crest-derived tissues, including bone, brain, and blood vessels, may also be affected in patients with NF1. Clinical manifestations of NF1 vasculopathy may include renal artery stenosis with consequent hypertension, occlusion resulting in cerebral or visceral infarcts, aneurysms resulting in haemorrhage, or arteriovenous fistulae (2). Most individuals with NF1-associated vascular lesions are asymptomatic (3), and the frequency of vasculopathy among NF1 patients in general has not been determined. Nevertheless, vascular disease appears to contribute to the excess mortality of young patients with NF1 (4).

Characteristic NF1 vascular lesions have been described in the entire arterial tree, but involvement of the renal arteries is most common (3, 5). NF1 vasculopathy of the cerebrum, endocrine system, gastrointestinal tract, and heart have also been reported (6). The lesions are patchy in distribution, but multiple vessels are frequently involved (3, 6–8).

Pathology of NF1 vasculopathy

Alterations of the vasculature occur within neurofibromas (9, 10), and a neurofibroma may invade or compress the walls of adjacent blood vessels (5, 6). These, however, are not the usual lesions of NF1 vasculopathy. The usual NF1 vascular lesions result from an intrinsic process involving the walls of arteries that are not directly associated with neurofibromas (5).

Reubi (11) first described the pathology of these lesions in 1945. Salyer and Salyer (12) classified them into four types according to the size of the vessel involved and the extent of the lesion: a *pure intimal* type, an *advanced intimal* type, the *intimal-aneurysmal* type, and the *nodular* (11) or *epithelial* (13) type. All are characterised by accumulations of cells in the intimal layer of the blood vessel, resulting in hyperplasia that narrows the lumen. Overlap occurs among the four types, and all are thought to involve the same pathogenesis at different stages of maturation (12, 14).

Neurofibromin expression

NF1 is a large gene spanning over 350 kb in the chromosomal region 17q11.2. The protein product,

neurofibromin, is composed of 2818 amino acids (15). Neurofibromin shares sequence and functional homology with a group of Ras-GTPase activating proteins (Ras-GAP family) (15–17). The Ras pathway transmits mitogenic signals to the nucleus, and neurofibromin acts as a negative regulator in this pathway. Loss of neurofibromin, therefore, produces increased mitogenic signalling, which leads to either cellular proliferation or differentiation (17, 18).

There is evidence that *NF1* functions as a tumour suppressor gene with somatic loss of the second *NF1* allele demonstrated in malignant peripheral nerve sheath tumours, juvenile chronic myelogenous leukaemia cells, pheochromocytomas, and some neurofibromas in NF1 patients (19, 20). Somatic loss of heterozygosity in an NF1 patient would usually be expected to produce complete loss of neurofibromin activity because most constitutional mutations of *NF1* are inactivating (21). However, haploinsufficiency at *Nf1* has also been shown to alter proliferation and survival in mast cells and melanocytes (22).

The *NF1* gene appears to have other functions that are unrelated to the Ras pathway. Neurofibromin associates with microtubules in some cell types (15, 16). The *NF1* homologue in *Drosophila* is involved in activation of an adenyl cyclase pathway that is required for growth, learning, and memory (23–25). This function may be important in humans as well (26).

The vessel wall

The blood vessel continuously responds to changes in its environment, and vascular remodelling and repair occur constantly. Intimal proliferation is an essential component of vascular remodelling and repair (27, 28). The intima is composed of a vascular endothelium, the innermost layer, and an elastic layer. The vascular endothelium exists as a single, continuous layer of cells acting as a structural and functional interface between the blood and the vessel wall (27, 29). Endothelial cells are metabolically active and have many physiological roles, including regulation of proliferation of the underlying smooth muscle cells (28). Dysfunction of the endothelium is thought to contribute to development of a number of pathologic entities, including atherosclerosis and hypertension (29, 30).

The internal elastic lamina is the endothelial basement membrane and forms the layer between the intima and media. The media is composed primarily of smooth muscle cells (SMCs) and extracellular matrix. The SMCs are pivotal in the development of intimal hyperplasia because they form the bulk of the cellular volume of the lesion and produce the extracellular matrix that makes up most of the intimal volume (28, 31).

There are two basic SMC phenotypes that represent the ends of a spectrum. The contractile phenotype is the differentiated state of normal adult vascular SMCs. At the opposite end of the spectrum is the SMC that functions almost exclusively in synthesis during development and repair. Following injury, SMCs undergo a phenotypic change as they migrate, proliferate, and synthesise matrix to repair the vessel wall. SMCs found in intimal hyperplastic lesions appear to be of the synthetic phenotype (27, 28, 31).

Theories of pathogenesis for NF1 vasculopathy

Salyer and Salyer (12) suggested that intimal thickening in NF1 vasculopathy is the result of proliferation of Schwann cells within the arteries. This implies a pathogenic relationship between these lesions and the neurofibromas that characterise this disease. More recent work has shown that the proliferating cells in NF1 vasculopathy are usually smooth muscle cells rather than Schwann cells (3, 5, 32), so Salyer and Salyer's interpretation seems unlikely to be correct in most instances.

Neurofibromin expression has been identified in the heart and in endothelial and smooth muscle cells of blood vessels (32–34). Type 2 neurofibromin is the predominant isoform expressed in endothelial (34) and vascular smooth muscle cells (32). Intrinsic expression of neurofibromin raises the possibility that NF1 vasculopathy results from a deficiency of neurofibromin function in the normal cellular components of the blood vessel wall.

Riccardi (35, 36) has suggested that NF1 vasculopathy results from a dysplastic process in which abnormal function of neurofibromin alters vascular histogenesis. This interpretation is not consistent with the observation that NF1 vasculopathy seems to be an acquired, rather than a congenital, abnormality in most cases. In the NF1 autopsy series examined by Salyer and Salyer (12), 5 of 8 individuals over 25 years of age, but only 2 of 10 patients who were younger than this, had lesions of NF1 vasculopathy. Development of new vascular lesions and progression of previously existing ones have been demonstrated in NF1 patients (37, 38).

Another possibility is that NF1 vasculopathy is caused by alteration of the normal process of vascular maintenance and repair. Riccardi (35, 36) has suggested that abnormal neurogenic wound healing with excessive proliferation of cells normally involved in repair underlies the development of many cutaneous neurofibromas in NF1 patients.

NF1 vascular lesions might arise in an analogous fashion if neurofibromin normally regulates blood vessel maintenance and repair. Vasculopathy might arise as a result of failure of the endothelium to suppress excessive SMC migration and proliferation or because of altered SMC response to normal endothelial signals (34).

Support for this hypothesis comes from studies of skin wound healing in heterozygous *Nf1* knockout mice. Neurofibromin expression occurs in normal fibroblasts and is upregulated during skin wound healing (39, 40). Skin wound healing by fibroblasts in heterozygous *Nf1* knockout mice is abnormal, with proliferation beyond the usual stage of cessation at wound maturation, as postulated for SMCs in NF1 vaculopathy.

NF1 vascular disease does not usually involve all arteries in an affected patient, although all presumably have the same constitutional mutation of the *NF1* gene. There are a number of possible explanations for this observation. For example, a 'second hit' mutation of the normal *NF1* allele may be needed to promote vascular changes. Alternatively, somatic mutation at another locus may be necessary for development of NF1 vascular lesions. Local injury to the vessel wall may also contribute to development of these lesions. External factors such as diet, smoking, exercise, and stress are known to play a role in atherosclerosis and may be involved in the development of NF1 vascular lesions as well.

The arterial distribution of NF1 vasculopathy is similar to the distribution observed in atherosclerosis. This distribution is thought to result from relatively low blood flow velocity and low and oscillating shear stress in the most frequently affected regions of the vasculature (27). Endothelial injury resulting from blood flow has been proposed as an important pathogenic factor in atherosclerosis (29) and may also explain the distribution of lesions in NF1 vasculopathy.

Methods are being developed to control the intimal hyperplasia that occurs in age-related vasculopathy and atherosclerosis (29, 41). As mediators of vascular hyperplasia are discovered, new pharmacologic interventions may become available to prevent or modify these structural and functional alterations. Targeting signalling pathways may prove to be a useful approach because cell migration and proliferation are critical events in the development of vasculopathy. Elucidation of the role of neurofibromin in endothelial dysfunction and smooth muscle cell migration and proliferation may lead to novel approaches to treatment of vascular disease in general as well as in patients with NF1.

References

1. Friedman JM, Riccardi VM. Clinical and epidemiological features. In: Friedman JM, Gutmann DH, MacCollin M, Riccardi VM, eds. Neurofibromatosis: Phenotype, Natural History and Pathogenesis, 3rd edition. Baltimore: Johns Hopkins University Press, 1999: 29–86.
2. Friedman JM. Vascular and endocrine abnormalities. In: Friedman JM, Gutmann DH, MacCollin M, Riccardi VM, eds. Neurofibromatosis: Phenotype, Natural History and Pathogenesis, 3rd edition. Baltimore: Johns Hopkins University Press, 1999: 274–296.
3. Lehrnbecher T, Gassel AM, Rauh V, Kirchner T, Huppertz H-I. Neurofibromatosis presenting as a severe systemic vasculopathy. Eur J Pediatr 1994: 153: 107–109.
4. Rasmussen S, Yang QH, Friedman JM. Mortality associated with neurofibromatosis 1 in the United States from 1983 to 1995: an analysis using data from death certificates. Am J Hum Genet 1999: 65: A49.
5. Greene JF, Fitzwater JE, Burgess J. Arterial lesions associated with neurofibromatosis. Am J Clin Path 1974: 62: 481–487.
6. Halpern M, Currarino G. Vascular lesions causing hypertension in neurofibromatosis. N Engl J Med 1965: 273 (5): 248–252.
7. Sobata E, Ohkuma H, Suzuki S. Cerebrovascular disorders associated with von Recklinghausen's neurofibromatosis: a case report. Neurosurgery 1988: 22: 544–549.
8. Muhonen MG, Godersky JC, VanGilder JC. Cerebral aneurysms associated with neurofibromatosis. Surg Neurol 1991: 36: 470–475.
9. Teixeira F, Martinez-Palomo A, Riccardi VM, Fernandez-Diez J. Vascular changes in cutaneous neurofibromas. Neurofibromatosis 1988: 1: 5–16.
10. Arbiser JL, Flynn E, Barnhill RL. Analysis of vascularity of human neurofibromas. J Am Acad Dermatol 1998: 38: 950–954.
11. Reubi F. Neurofibromatose et lésions vasculares. Schweiz Med Wochenschr 1945: 75: 463–465.
12. Salyer WR, Salyer DC. The vascular lesions of neurofibromatosis. Angiology 1974: 25: 510–519.
13. Feyrter F. Über die vasculare Neurofibromatose, nach Utersuchungen am menschlichen Magen-Darmschlauch. Virchow Arch Pathol Anat 1949: 317: 221–265.
14. Huffman JL, Gahtan V, Bowers VD, Mills JL. Neurofibromatosis and arterial aneurysms. Am Surg 1996: 62: 311–314.
15. Shen MH, Harper PS, Upadhyaya M. Molecular genetics of neurofibromatosis type 1 (NF1). J Med Genet 1996: 33: 2–17.
16. Feldkamp MM, Gutmann DH, Guha A. Neurofibromatosis type 1: piecing the puzzle together. Can J Neurol Sci 1998: 25: 181–191.
17. Weiss B, Bollag G, Shannon K. Hyperactive Ras as a therapeutic target in neurofibromatosis type 1. Am J Med Genet 1999: 89: 14–22.
18. Gabriel KR. Neurofibromatosis. Curr Opin Pediatr 1997: 9: 89–93.
19. Korf BR. Neurofibromas and malignant tumors of the peripheral nerve sheath. In: Friedman JM, Gutmann DH, MacCollin M, Riccardi VM, eds. Neurofibromatosis: Phenotype, Natural History and Pathogenesis, 3rd edition. Baltimore: Johns Hopkins University Press, 1999: 142–161.
20. Gutmann DH, Gurney JG. Other malignancies. In: Friedman JM, Gutmann DH, MacCollin M, Riccardi VM, eds. Neurofibromatosis: Phenotype, Natural History and

Pathogenesis, 3rd edition. Baltimore: Johns Hopkins University Press. 1999: 231–249.
21. Upadhyaya M, Shaw DJ, Harper PS. Molecular basis of neurofibromatosis type 1 (NF1): mutation analysis and polymorphisms in the *NF1* gene. Hum Mut 1994: 4: 83–101.
22. Ingram DA, Yan FC, Travers JB, Wenning MJ, Hiatt K, New S, Hood A, Shannon K, Williams DA, Clapp DW. Genetic and biochemical evidence that haploinsufficiency of the Nf1 tumour suppressor gene modulates melanocyte and mast cell fates *in vivo*. J Exp Med 2000: 191: 181–187.
23. The I, Hannigan GE, Cowley GS, Reginald S, Zhong Y, Gusella JF, Hariharan IK, Bernards A. Rescue of a *Drosophila* NF1 mutant phenotype by protein kinase A. Science 1997: 276: 791–794.
24. Guo HF, The I, Hannan F, Bernards A, Zhong Y. Requirement of Drosophila NF1 for activation of adenylyl cyclase by PACAP38-like neuropeptides. Science 1997: 276: 795–798.
25. Guo HF, Tong J, Hannan F, Luo L, Zhong Y. A neurofibromatosis-1-regulated pathway is required for learning in *Drosophila*. Nature 2000: 403: 895–898.
26. Fahsold R, Hoffmeyer S, Mischung C, Gille C, Ehlers C, Kucukceylan N, Abdel-Nour M, Gewies A, Peters H, Kaufmann D, Buske A, Tinschert S, Nurnberg P. Minor lesion mutation spectrum of the entire *NF1* gene does not explain its high mutability but points to a functional domain upstream of the GAP-related domain. Am J Human Genet 2000: 66: 790–818.
27. Kraiss L, Clowes A. Response of the arterial wall to injury and intimal hyperplasia. In: Sidawy AN, Sumpio BE, DePalma RG, eds. The Basic Science of Vascular Disease. New York: Futura Publishing Company, 1997: 289–319.
28. Desmouliere A, Gabbiani G. Smooth muscle cell and fibroblast biological functional features. In: Schwartz SM, Mecham RP, eds. The Vascular Smooth Muscle Cell: Molecular and Biological Responses to the Extracellular Matrix. London: Academic Press, Inc., 1995: 329–348.
29. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 1993: 362: 801–809.
30. Cooper LT, Cooke JP, Dzau VJ. The vasculopathy of aging. Journal of Gerontology 1994: 49 (5): B191–B196.
31. Hungerford JE, Little CD. Developmental biology of the vascular smooth muscle cell: Building a multilayered vessel wall. J Vasc Res 1999: 36: 2–27.
32. Ahlgren-Beckendorf JA, Maggio WW, Chen F, Kent TA. Neurofibromatosis 1 mRNA expression in blood vessels. Biochem Biophys Res Commun 1993: 197: 1019–1024.
33. Brannan CI, Perkins AS, Vogel KS, Ratner N, Nordlund ML, Reid SW, Buchberg AM, Jenkins NA, Parada LF, Copeland NG. Targeted disruption of the neurofibromatosis type-1 gene leads to developmental abnormalities of the heart and various neural crest-derived tissues. Genes Develop 1994: 8: 1019–1029.
34. Norton KK, Xu J, Gutmann DH. Expression of the neurofibromatosis 1 gene product, neurofibromin, in blood vessel endothelial cells and smooth muscle. Neurobiol Dis 1995: 2: 13–21.
35. Riccardi VM. Histogenesis control genes and neurofibromatosis 1. Eur J Pediatrics 2000: 159: 475–476.
36. Riccardi VM. Neurofibromatosis: Phenotype, Natural History and Pathogenesis, 2nd edition. Baltimore: Johns Hopkins University Press, 1992.
37. Kurien A, John PR, Milford DV. Hypertension secondary to progressive vascular neurofibromatosis. Arch Dis Child 1997: 76: 454–455.
38. Pentecost M, Stanley P, Takahashi M, Isaacs H Jr. Aneurysms of the aorta and subclavian and vertebral arteries in neurofibromatosis. Am J Dis Child 1981: 135: 475–477.
39. Atit RP, Crowe MJ, Greenhalgh DG, Wenstrup RJ, Ratner N. The *Nf1* tumour suppressor regulates mouse skin wound healing, fibroblast proliferation, and collagen deposited by fibroblasts. J Invest Dermat 1999: 112 (6): 835–842.
40. Yla-Outinen H, Aaltonen V, Bjorkstrand AS, Hirvonen O, Lakkakorpi J, Vaha-Kreula M, Laato M, Peltonen J. Upregulation of tumor suppressor protein neurofibromin in normal human wound healing and in vitro evidence for platelet derived growth factor (PDGF) and transforming growth factor-B1 (TGF-β1) elicited increase in neurofibromin mRNA steady-state levels in dermal fibroblasts. J Invest Derm 1998: 110 (3): 232–237.
41. Phillips-Hughes J, Kandarpa K. Restenosis: pathophysiology and preventive strategies. J Vasc Interven Radiol 1996: 7 (3): 321–333.

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Cardiac Findings in an Individual With Neurofibromatosis 1 and Sudden Death

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Vascular lesions in neurofibromatosis 1 (NF1) are infrequently recognised as manifestations of the disease, yet they can produce serious complications. Most individuals with NF1 vasculopathy are asymptomatic, which may contribute to underestimation of its frequency. A recent study indicates that vascular changes in individuals with NF1 contribute to mortality at younger ages. We report the sudden death of a young man with NF1. On autopsy examination there was evidence of an intramyocardial vasculopathy characteristic of the vascular pathology previously described in NF1. Other cardiac findings included non-specific cardiomyopathic changes, myocardial fibrosis, and a "floppy" mitral valve. © 2001 Wiley-Liss, Inc.

KEY WORDS: NF1; neurofibromin; vasculopathy

INTRODUCTION

Neurofibromatosis 1 (NF1) affects 1 in 3,700 people, making it one of the most common inherited diseases [Friedman, 1999]. NF1 is transmitted as an autosomal-dominant condition with variable expression. The most frequent clinical manifestations include café-au-lait spots and dermal neurofibromas, which result from abnormalities in neural crest derived cell types such as melanocytes and Schwann cells. The NF1 gene product, neurofibromin, is thought to be a tumour suppressor [Ponder, 1990]. It functions as a negative growth regulator in certain cell types [Shen et al., 1996], yet other functions are likely [Feldkamp et al., 1998]. High levels of neurofibromin expression are found in neural crest derived cell types, but neurofibromin expression has also been detected in non-neural crest derived tissues, such as the heart and endothelial and smooth muscle cells of blood vessels [Ahlgren-Beckendorf et al., 1993; Brannan et al., 1994; Norton et al., 1995].

Lesions in the blood vessels of individuals with NF1 represent a potentially important complication of the disease. Characteristic vascular lesions in NF1 patients have been described in the entire arterial tree, although the renal arteries are most commonly involved [Greene et al., 1974; Lehrnbecher et al., 1994]. Venous involvement in NF1 vasculopathy has rarely been reported [Lehrnbecher et al., 1994; Finley and Dabbs, 1998].

Clinical manifestations of NF1 vasculopathy may include hypertension, usually as a result of renal artery stenosis [Halpern and Currarino, 1965; Faggioli et al., 1992; Kurien et al., 1997; Finley and Dabbs, 1998] or thoracic aortic coarctation [Rowen et al., 1975; Donaldson et al., 1985]. Other reported complications are occlusions resulting in cerebral and visceral infarcts [Levisohn et al., 1978; Taboada et al., 1979; Sobata et al., 1988; Woody et al., 1992], aneurysms resulting in haemorrhage [Muhonen et al., 1991; Huffman et al., 1996; Griffiths et al., 1998; Singh et al., 1998], and arteriovenous fistula [Murayama et al., 1999]. Convulsions, hemiparesis [Piovesan et al., 1999], and headaches may also be clinical manifestations of NF1 vasculopathy, but the range of clinical manifestations is not well characterised. The frequency of NF1 vasculopathy is unknown. It is diagnosed clinically in only a small fraction of NF1 patients. However, most individuals with NF1 associated vascular lesions are asymptomatic [Lehrnbecher et al., 1994], so the actual prevalence may be much higher. Salyer and Salyer [1974] found vascular lesions in seven of 18 individuals with NF1 studied at autopsy.

We report a previously healthy 33-year-old man with NF1 who died suddenly. Autopsy examination revealed multiple cardiac abnormalities, including a vasculopathy that likely contributed to his untimely death.

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CASE REPORT

A 33-year-old man presented to the British Columbia Provincial Medical Genetics Programme for evaluation of possible neurofibromatosis. He reported having brown spots on his skin for as long as he could remember, and lumps on his skin since he was a child. The dermal nodules had increased in number with age. He had always enjoyed good health, apart from chronic sinusitis, which required surgery. He had intermittent swelling and pain of his knees related to a hockey injury. He did not have skeletal problems unrelated to injuries. He reported experiencing occasional headaches that usually resolved without treatment. There was no history of vision or hearing problems, and he did not complain of chest pain, palpitations, or distress.

No one else in his family is known to have any features suggestive of neurofibromatosis; however, other family members were not examined. The family history was negative for cardiovascular disease. The patient had no children.

On physical examination, the patient was a well developed, muscular, alert, and active 33-year-old oriental man in no distress. His height was 178 cm, weight was 78 kg, and head circumference was 61 cm (4 standard deviations above mean). His blood pressure was 150/90 mmHg. He had at least 12 typical café-au-lait spots larger than 1.5 cm in diameter, as well as axillary and inguinal freckling. He had dozens of subcutaneous tumours, ranging in size from a few millimetres to about 2.5 cm, and a few small cutaneous tumours on the trunk, arms, and face. He did not have physical signs suggestive of either Noonan syndrome or Watson syndrome. His physical examination was otherwise normal.

An ophthalmology examination with slit lamp was suggested, as was routine monitoring of his blood pressure to determine if he had hypertension. However, neither was initiated, as he died nine days later during a game of ice hockey.

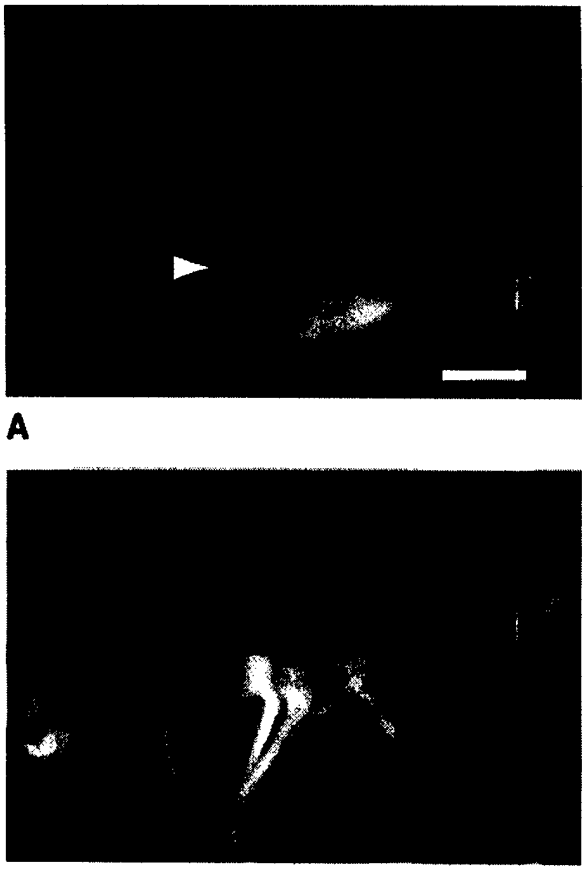

Fig. 1. Photographs of the heart at autopsy. (**A**, upper image) Transverse slice of left and right ventricles. Arrowhead indicates areas of fibrosis in the left ventricle. Bar = 1 cm. (**B**, lower image) Mitral valve showing interchordal hooding (Arrowhead) with mild thickening and ballooning of leaflets toward the left atrium.

PATHOLOGY

A complete autopsy was performed. External examination confirmed the clinically noted features of NF1. The remainder of the external examination was normal. Internal examination revealed several abnormalities of the heart.

Overall configuration of the heart and accompanying great vessels was normal. The heart was mildly hypertrophied (450 g, normal range 296–382 g for a person his size), with minimal dilation of right and left ventricles. The left ventricular free wall and septum were of uniform thickness. There was no evidence of a recent myocardial infarction. However, areas of fibrosis were noted in the left ventricular myocardium (Fig. 1A). The fibrosis was located predominantly in the inner one-half of the posteromedial and anteromedial myocardium, and was most prominent in the middle to apical portions of the heart. Apically, the fibrosis became somewhat circumferential in distribution. In addition, a more generalised mild interstitial and perivascular fibrosis was present. Focally, the fibrosis became accentuated and was accompanied by disarray of cardiac myocytes (Fig. 2A).

The epicardial coronary arteries arose normally in a right-dominant manner. Approximately 4 to 5 cm from its point of origin, the left anterior descending coronary artery was severely narrowed by an eccentrically located fibrotic, hypocellular, and lipid-poor plaque. No thrombosis or dissection was observed in the epicardial coronary arteries. Intramyocardial coronary arteries were mildly thickened. Additionally, an occasional intramyocardial coronary artery showed significant narrowing by a highly cellular intimal proliferation (Fig. 2B).

The mitral valve showed mild to moderate interchordal hooding with mild ballooning of the distal half of the leaflets toward the left atrium (Fig. 1B). These changes, which primarily affected the anterior leaflet, were accompanied by mild thickening of the leaflet tissues with expansion of the spongiosa and focal

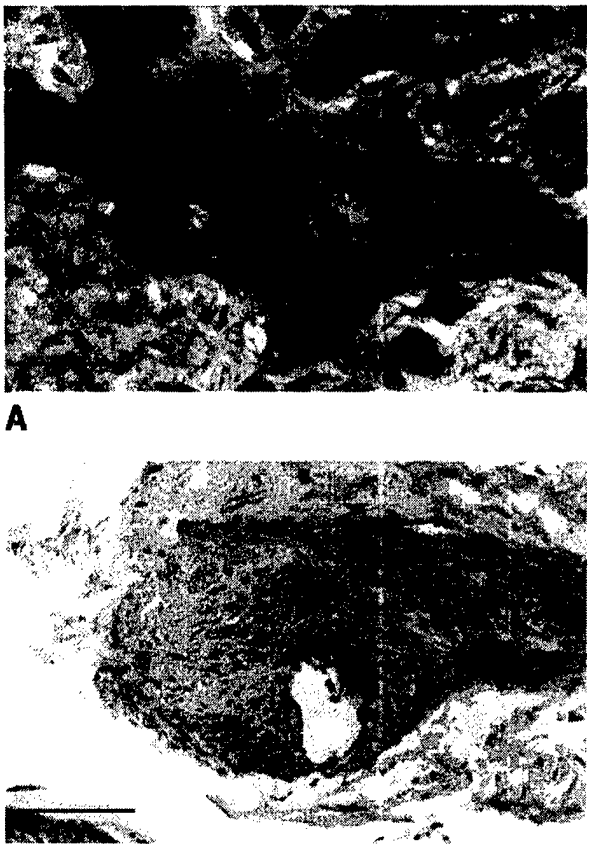

Fig. 2. Representative photomicrographs of the heart. (A, upper image) Myocardium showing disarray of myocytes (Arrowhead) and interstitial fibrosis (Arrow)(Masson Trichrome, Bar = 100λM). (B, lower image) Intramyocardial coronary artery with intimal proliferation (Arrow) causing significant stenosis of the arterial lumen. (Hematoxylin and Eosin, Bar = 100λM).

effacement of the leaflet fibrosa. Mild to moderate thickening of chordae tendinae was also observed, with focal changes suggesting mild chordal redundancy. Other valves showed no significant pathologic abnormalities.

No evidence of pheochromocytoma, renal artery stenosis, coarctation of the aorta, or other pathologic lesions was observed. The autopsy examination was otherwise normal. Death was attributed to cardiac dysrhythmia resulting from one or more of these cardiac anomalies.

DISCUSSION

This young man with NF1, who previously had no evidence of cardiovascular disease, collapsed while playing hockey. Autopsy revealed a number of cardiac abnormalities. First, the proximal left anterior descending coronary artery was severely narrowed by a fibrotic, lipid-poor plaque that likely represents a manifestation of atherosclerosis. Second, the intramyocardial arteries were focally severely narrowed by cellular intimal proliferation. Third, the heart was hypertrophied, and the myocardium showed a generalised interstitial and perivascular fibrosis that was focally accentuated and associated with myocyte disarray, findings indicative of a cardiomyopathic process. Fourth, the mitral valve was abnormal, with an appearance resembling a "floppy" mitral valve. No other disease process was evident on autopsy. Each of the patient's cardiovascular findings has been associated individually with NF1 [Salyer and Salyer, 1974; Halper and Factor, 1984; Fitzpatrick and Emanuel, 1988; Scotto di Uccio et al., 1988]. However, the abnormalities present in the heart of this young man have not previously been reported *together* in an individual with NF1. The distinctive vasculopathy in this individual, combined with the presence of other cardiovascular findings that have previously been described in autopsies of NF1 patients [Halper and Factor, 1984; Scotto di Uccio et al., 1988; Daly and Rubinstein, 1992], seems much more likely to be a manifestation of his NF1 than an unrelated chance occurrence.

Sudden death in NF1 is rare. Fatal haemorrhage due to rupture of an artery affected by NF1 vasculopathy has been described [Miura et al., 1997; Griffiths et al., 1998]. Infiltration or compression of arteries by neurofibromas or tumours has also led to fatal haemorrhage in NF1 patients [Leier et al., 1972; Keenan, 1982; Brady and Bolan, 1984]. Other causes of sudden death in NF1 patients include an intrathoracic neurofibroma postulated to have affected vagus nerve function [Chow et al., 1993], and an occult glioblastoma multiforme [Unger et al., 1984]. Hypertrophic cardiomyopathy has been reported in patients with NF1 [Rosenquist et al., 1970; Elliott et al., 1976; Sachs et al., 1984; Salvador et al., 1986; Fitzpatrick and Emanuel, 1988] and is a recognised cause of sudden death in the general population [Mukhtar and Ihunwo, 1999].

Reubi first described vascular lesions in association with NF1 in 1945. He postulated that all individuals with NF1 have vascular involvement, but that it goes undetected because of an absence of symptoms [Reubi, 1945]. Autopsy studies of confirmed NF1 cases have shown that NF1 vasculopathy is common, but they do not support Reubi's hypothesis that all individuals with NF1 have vascular involvement [Salyer and Salyer, 1974]. However, the frequency of this manifestation, like that of most disease manifestations of NF1, may depend on age.

NF1 vasculopathy has been observed most often in the renal arteries, but involvement of the arteries of the cerebrum, endocrine system, gastrointestinal tract [Halpern and Currarino, 1965], pulmonary system [Samuels et al., 1999], retina [Moadel et al., 1994; Tholen et al., 1998], and heart [Halper and Factor, 1984; Kandarpa et al., 1988; Nogami et al., 1991; Daly and Rubinstein, 1992; Fuchi et al., 1997] has also been reported. Salyer and Salyer [1974] described four types of vascular lesions according to the size of the vessel involved and the extent of the lesion. These small vessel lesions do not result from external compression by a

neurofibroma but rather from an intrinsic process that involves the walls of the arteries themselves.

In the present case, only a few intramyocardial coronary arteries were affected by the vasculopathy described by Salyer and Salyer [1974], although all of the arteries presumably harboured the same constitutional mutation of the *Nf1* gene. This observation raises the possibility that other factors such as a "second hit" mutation of the normal *Nf1* allele, a mutation at another locus, or a local injury might be necessary for the vasculopathy to develop.

Over 50 years have passed since Reubi [1945] first described lesions in the vessels of individuals with NF1, but the pathogenesis of NF1 vasculopathy remains unclear. The intimal thickening was originally thought to result from proliferation of Schwann cells within the arteries [Salyer and Salyer, 1974], but more recent work has shown the proliferating cells in these lesions to be smooth muscle, not Schwann cells [Lehrnbecher et al., 1994; Finley and Dabbs, 1998]. Animal models demonstrate that neurofibromin expression does occur in endothelial and smooth muscle cells of blood vessels [Ahlgren-Beckendorf et al., 1993; Norton et al., 1995].

Neurofibromin expression has also been detected in mouse fibroblasts [Atit et al., 1999] and is upregulated during skin wound healing in normal fibroblasts [Yla-Outinen et al., 1998]. Mutations in the *Nf1* gene cause a reduction of neurofibromin upregulation in fibroblasts that presumably results in extended mitogenic effects [Atit et al., 1999]. Decreased neurofibromin expression in the blood vessels of NF1 patients may also lead to extended mitogenic effects and NF1 vasculopathy [Hamilton and Friedman, 2000].

A recent study has shown that vascular changes contribute to mortality in NF1 patients, but only at younger ages [Rasmussen et al., 1999]. It is important for clinicians to recognise vascular lesions as a potentially serious feature of NF1 so that appropriate management can be provided.

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REFERENCES

Ahlgren-Beckendorf JA, Maggio WW, Chen F, Kent TA. 1993. Neurofibromatosis 1 mRNA expression in blood vessels. Biochem Biophys Res Commun 197:1019–1024.

Atit RP, Crowe MJ, Greenhalgh DG, Wenstrup RJ, and Ratner N. 1999. The *Nf1* tumour suppressor regulates mouse skin wound healing, fibroblast proliferation, and collagen deposited by fibroblasts. J Invest Dermatol 112:835–842.

Brady DB, Bolan JC. 1984. Neurofibromatosis and spontaneous hemothorax in pregnancy: two case reports. Obstet Gynecol 63 (suppl):35S.

Brannan CI, Perkins AS, Vogel KS, Ratner N, Nordlund ML, Reid SW, Buchberg AM, Jenkins NA, Parada LF, Copeland NG. 1994. Targeted disruption of the neurofibromatosis type-1 gene leads to developmental abnormalities in heart and various neural crest-derived tissues. Genes Develop 8:1019–1029.

Chow LT, Shum BS, Chow WH. 1993. Intrathoracic vagus nerve neurofibroma and sudden death in a patient with neurofibromatosis. Thorax 48:298–299.

Daly MP, and Rubinstein MN. 1992. A case of neurofibromatosis associated with coronary artery aneurysm and myocardial infarction. Clin Cardiol 15:616–618.

Donaldson MC, Ellison LH, Ramsby Gr. 1985. Hypertension from isolated thoracic coarctation associated with neurofibromatosis. J Pediatr Surg 20:169–171.

Elliott CM, Tajik AJ, Giuliani ER, Gorndon H. 1976. Idiopathic hypertrophic subaortic stenosis associated with cutaneous neurofibromatosis: report of a case. Am Heart J 92:368–372.

Faggioli GL, Gargiulo M, Bertoni F, Tarantini S, Stella A. 1992. Hypertension due to an aneurysm of the left renal artery in a patient with neurofibromatosis. Ann Vasc Surg 6:456–459.

Feldkamp MM, Gutmann DH, Guha A. 1998. Neurofibromatosis type 1: piecing the puzzle together. Can J Neurol Sci 25:181–191.

Finley JL, Dabbs DJ. 1998. Renal vascular smooth muscle proliferation in neurofibromatosis. Hum Pathol 19:107–110.

Fitzpatrick AP, Emanuel RW. 1988. Familial neurofibromatosis and hypertrophic cardiomyopathy. Br Heart J 60:247–251.

Friedman JM. 1999. Epidemiology of neurofibromatosis type 1. Am J Med Genet 89:1–6.

Fuchi T, Ishimoto N, Kajinami T, Kajinami M, Ohmichi N, Kinoshita M. 1997. A 23 year old patient with Neurofibromatosis associated with acuite myocardial infarction, vasospasm and a coronary artery ectasis. Intern Med 36(9):618–623.

Greene JF, Fitzwater JE, Burgess J. 1974. Arterial lesions associated with neurofibromatosis. Am J Clin Pathol 62:481–487.

Griffiths AP, White J, Dawson A. 1998. Spontaneous haemothorax: a cause of sudden death in von Recklinghausen's disease. Postgrad Med J 74:679–681.

Halper J, Factor S. 1984. Coronary lesions in neurofibromatosis associated with vasospasm and myocardial infarction. Am Heart J 108:420–422.

Halpern M, Currarino G. 1965. Vascular lesions causing hypertension in neurofibromatosis. N Engl J Med 273:248–252.

Hamilton SJ, Friedman JM. 2000. Insights into the pathogenesis of neurofibromatosis 1 vasculopathy. Clin Genet 58:341–344.

Huffman JL, Gahtan V, Bowers VD, Mills JL. 1996. Neurofibromatosis and arterial aneurysms. Am Surg 62:311–314.

Kandarpa K, Stoll JF, Reiss C, Rutherford JD, Cohn LM. 1988. A case of neurofibromatosis associated with coronary artery aneurysm and myocardial infarction. Cardiovasc and Intervent Radiol 11:143–145.

Keenan RA, Robinson DJ, Briggs PC. 1982. Fatal spontaneous retroperitoneal hemorrhage caused by von Recklinghausen's neurofibromatosis. J R Coll Surg Edinb 27:310.

Kurien A, John PR, and Milford DV. 1997. Hypertension secondary to progressive vascular neurofibromatosis. Arch Dis Child 76:454–455.

Lehrnbecher T, Gassel AM, Rauh V, Kirchner T, Huppertz H-I. 1994. Neurofibromatosis presenting as a severe systemic vasculopathy. Eur J Pediatr 153:107–109.

Leier CV, DeWan CJ, Anatasia LF. 1972. Fatal hemorrhage as a complication of neurofibromatosis. Vasc Surg 6:98–101.

Levisohn PM, Mikhael MA, Rothman SM. 1978. Cerebrovascular changes in neurofibromatosis. Dev Med Child Neurol 20:789–793.

Miura H, Taira O, Uchida O, Usuda J, Hirai S, Kato H. 1997. Spontaneous hemothorax associated with von Recklinghausen's disease: review of occurrence in Japan. Thorax 52:577–578.

Moadel K, Yannuzzi LA, Ho AC, Ursekar A. 1994. Retinal vascular occlusive disease in a child with neurofibromatosis. Arch Ophthalmol 112:1021–1023.

Muhonen MG, Godersky JC, VanGilder JC. 1991. Cerebral aneurysms associated with neurofibromatosis. Surg Neurol 36:470–475.

Mukhtar AU, Ihunwo AO. 1999. Hypertrophic cardiomyopathy: case report. East Afr Med J 76:535–536.

Murayama Y, Usami S, Abe T, Hata Y, Ganaha F, and Massoud TF. 1999. Transvenous doppler guidewire sonographic monitoring during

treatment of a complex vertebral arteriovenous fistula associated with neurofibromatosis type 1. Neuroradiology 41:328–333.

Nogami A, Hiroe M, Marumo F. 1991. Regional sympathetic denervation in von Recklinghausen's disease with coronary spasm and myocarditis. Int J Cardiol 32:397–400.

Norton KK, Xu J, Gutmann DH. 1995. Expression of the neurofibromatosis 1 gene product, neurofibromin, in blood vessel endothelial cells and smooth muscle. Neurobiol Dis 2:13–21.

Piovesan EJ, Scola RH, Werneck LC, Zetola VHF, Novak EM, Iwamoto FM, Piovesan LM. 1999. Neurofibromatosis, stroke and basilar impression. Arq Neuropsiquiatr 57:484–488.

Ponder B. 1990. Neurofibromatosis gene cloned. Hum Genet 346:703–704.

Rasmussen S, Yang QH, Friedman JM. 1999. Mortality associated with neurofibromatosis 1 in the United States from 1983 to 1995: an analysis using data from death certificates. Am J Hum Genet 65:A49.

Reubi F. 1945. Neurofibromatosis et lesions vasculares. Schweiz Med Wochenschr 75:463–465.

Rosenquist GC, Krovetz LJ, Haller JA, Simon AL, Bannayan GA. 1970. Acquired right ventricular outflow obstruction in a child with neurofibromatosis. Am Heart J 79:103–108.

Rowen M, Dorsey TJ, Kegel S, Ostermiller WE. 1975. Thoracic coarctation associated with neurofibromatosis. Am J Dis Child 129:113–115.

Sachs RN, Buschauer-Bonnet Ch, Kemeny JL, Amouroux J, Lanfranchi J. 1984. Myocardiopathie hypertrophique et maladie de von recklinghausen. Rev De Med Interne 5:154–156.

Salvador A, Bigi R, Corradetti C, Occhi G, Partesana N, Zatta G, Tarolo GL. 1986. Malattia di Recklinghausen e cardiomiopatia Ipertrofica. Minerva Cardioangiol 34:771–773.

Salyer WR, Salyer DC. 1974. The vascular lesions of neurofibromatosis. Angiology 25:510–519.

Samuels N, Berkman N, Milgalter E, Bar-Ziv J, Amir G, Kramer MR. 1999. Pulmonary hypertension secondary to neurofibromatosis: intimal fibrosis versus thromboembolism. Thorax 54:858–859.

Scotto di Uccio V, Petrillo C, Chiosso M, de Tommasis L. 1988. Mitral valve prolapse in recklinghausen's disease. Description of a case. Minerva Cardioangiol 36:331–333.

Shen MH, Harper P, Upadhyaya M. 1996. Molecular genetics of neurofibromatosis type 1 (NF1). J Med Genet 33:2–17.

Singh S, Riaz M, Wilmshurst AD, Small JO. 1998. Radial artery aneurysm in a case of neurofibromatosis. Br J Plast Surg 51:564–566.

Sobata E, Ohkuma H, Suzuki S. 1988. Cerebrovascular disorders associated with von Recklinghausen's neurofibromatosis: a case report. Neurosurgery 22:544–549.

Taboada D, Alonso A, Moreno J, Muro D, Mulas F. 1979. Occlusion of the cerebral arteries in Recklinghausen's disease. Neuroradiology 18:281–284.

Tholen AM, Messmer EP, Landau K. 1998. Peripheral retinal vascular occlusive disorder in a young patient with neurofibromatosis 1. J Retinal and Vitreous Diseases 18:184–186.

Unger PD, Taff ML, Song S, Schwartz IS. 1984. Sudden death in a patient with Von Recklinghausen's neurofibromatosis. Am J Forensic Med Pathol 5:175–179.

Woody RC, Perrot LJ, Beck SA. 1992. Neurofibromatosis cerebral vasculopathy in an infant: clinical, neuroradiographic, and neuropathologic studies. Pediatr Pathol 12:613–619.

Yla-Outinen HY, Aaltonen V, Bjorkstrand A. 1998. Upregulation of tumor suppressor protein neurofibromin in normal wound healing and in vitro evidence for platelet derived growth factor (P13GF) and transforming growth factor-beta 1 (TGF-1) elicited increase in neurofibromin mRNA steady-state levels in dermal fibroblasts. J Invest Dermatol 110:232–237.

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Cardiovascular Malformations and Other Cardiovascular Abnormalities in Neurofibromatosis 1

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Although it is well recognized that a peripheral vasculopathy may occur in patients with neurofibromatosis 1 (NF1), it is unclear whether cardiovascular abnormalities are more common. We reviewed the frequency of cardiovascular abnormalities, in particular, cardiovascular malformations (CVMs), among 2322 patients with definite NF1 in the National Neurofibromatosis Foundation International Database from 1991–98. Cardiovascular malformations were reported in 54/2322 (2.3%) of the NF1 patients, only 4 of whom had Watson syndrome or NF1-Noonan syndrome. There was a predominance of Class II "flow" defects [Clark, 1995: Moss and Adams' Heart Disease in Infants, Children, and Adolescents Including the Fetus and Young Adult. p 60–70] (43/54, 80%) among the NF1 patients with CVMs. Pulmonic stenosis, that was present in 25 NF1 patients, and aortic coarctation, that occurred in 5, constitute much larger proportions of all CVMs than expected. Of interest was the paucity of Class I conotruncal defects (2 patients with tetralogy of Fallot), and the absence of atrioventricular canal, anomalous pulmonary venous return, complex single ventricle and laterality defects. Besides the 54 patients with CVMs, there were 27 patients with other cardiac abnormalities (16 with murmur, 5 with mitral valve prolapse, 1 with intracardiac tumor, and 5 with electrocardiogram abnormalities). No patient in this study had hypertrophic cardiomyopathy. There were 16 patients who had a peripheral vascular abnormality without an intracardiac CVM, plus an additional 4 patients among those with a CVM who also had a peripheral vascular abnormality. Am. J. Med. Genet. 95: 108–117, 2000. © 2000 Wiley-Liss, Inc.

KEY WORDS: cardiovascular malformation; congenital heart defect; database; hypertrophic cardiomyopathy; neurofibromatosis type 1; NF1; NF1-Noonan

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syndrome; peripheral arterial stenosis; pulmonic stenosis; Watson syndrome

INTRODUCTION

Neurofibromatosis 1 (NF1) is a distinctive autosomal dominant disorder (MIM 162200) with multisystem involvement [Rubenstein and Korf, 1990; Huson and Hughes, 1994; Friedman et al., 1999]. The type and frequency of cardiovascular abnormalities is unclear, although some individuals with NF1 have a vasculopathy consisting of peripheral arterial stenoses and ectasias [Rubenstein and Korf, 1990; Riccardi, 1992; Huson and Hughes, 1994; Friedman, 1999].

The type and incidence of cardiovascular malformations (CVMs) in NF1 have not been well-defined [Lin and Garver, 1988; Friedman and Birch, 1997; Friedman, 1999]. The frequency of CVMs in 8 large series of NF1 patients ranged from 0.4–6.4% (Table I). Often, it was unclear whether the diagnosis of a CVM was well established in these patients, whether the diagnosis of NF1 was certain, or whether a related dysmorphic syndrome (Watson, NF1-Noonan) was present. Despite the vagaries of reporting, there is a predominance of pulmonic stenosis, often valvar, in these series. Likewise, smaller series and individual case reports frequently noted pulmonic stenosis. The single "NF" patient reported with a complex CVM [Stoll et al., 1995] did not have NF1 substantiated and may have had a multiple congenital anomaly syndrome related to parental consanguinity.

It might be hypothesized that the only NF1 patients with cardiac abnormalities are those with the Watson syndrome or NF1-Noonan syndrome. Pulmonic stenosis is one of the cardinal clinical features of Watson syndrome [Watson, 1967; Allanson et al., 1991] and may occur in patients with the NF1-Noonan syndrome [Sharland et al., 1992], although most patients with NF1-Noonan syndrome do not have a CVM.

Although Watson syndrome is an allelic variant of NF1, the molecular basis of NF1-Noonan syndrome seems diverse. Several cases of the NF1-Noonan syndrome result from an *NF1* mutation [Tassabehji et al., 1993; Kayes et al., 1994; Stern et al., 1995; Colley et al., 1996], but most cases of Noonan syndrome do not have NF1. Noonan syndrome is a distinct disorder (with a gene locus at 12q22 in some families), and valvar pulmonic stenosis is also the most common CVM in this condition [Sharland et al., 1992; Marino et al., 1999]. Further cardiac overlap with NF1 is suggested by the occurrence of hypertrophic cardiomyopathy in approximately 10–20% of patients with Noonan syndrome [Sharland et al, 1992; Marino et al., 1999].

A third group of NF1 patients who might be considered more likely to have a CVM are those with large deletions. They often have dysmorphic features and mental retardation, and 3 have been reported with a CVM [Leppig et al., 1997; Wu et al., 1995; Wu et al., 1997; Tonsgard et al., 1997; Riva et al., 2000]. All of these patients (Watson syndrome, NF1-Noonan syndrome, *NF1* microdeletion syndrome) are part of the NF1 population, and their association with CVMs provides evidence that *NF1* mutations can cause CVMs.

Less frequent than CVM are reports of hypertrophic cardiomyopathy (HCM) in NF1 patients (Table II). Miscellaneous cardiac abnormalities that have been reported in association with NF1 (Table III) include assorted intracardiac tumors, mitral valve prolapse and aortic dilation. Strictly speaking, these latter two abnormalities may be regarded as valve and arterial dysplasia distinct from cardiac malformations.

For this analysis of CVMs and other cardiac abnormalities in patients with NF1, we used the National Neurofibromatosis Foundation International Database (NNFFID), an international multicenter collaborative system for collecting demographic information, descriptions of signs and symptoms, basic measurements, and certain psychological information on individuals and families with NF [Friedman et al., 1993]. The NNFFID was established and is maintained by the National Neurofibromatosis Foundation. Twenty-four clinics throughout the world voluntarily enter data into the NNFFID. Any qualified investigator can access these data for use in appropriate studies by contacting the authors.

A preliminary analysis of data from the NNFFID suggested that CVMs, especially pulmonic stenosis, are more common in NF1 [Friedman and Birch, 1998]. We present further descriptive analysis of 97 patients with verified NF1 contributed to the NNFFID between 1991 and 1998.

PATIENTS AND METHODS

As described previously [Friedman et al., 1993], the NNFF International Database collects data on 98 items and allows for serial collection. All patients in this analysis had NF1 as defined by the NIH Consensus Conference [1988]. We tried to determine whether any patients had been previously reported. We identified the syndromic patients reported to have possible Watson or NF1-Noonan syndrome and took special note of them in the analysis. The diagnoses are those provided by the contributors. In some instances, the referring NF center was queried to provide additional information, but the NF clinic records rarely contained more information on the CVM than was reported to the database initially.

To promote insight into possible mechanisms and timing, cardiac abnormalities were categorized as CVMs, other non-CVM abnormalities, and peripheral vascular abnormalities [Lin and Pexeider, 1994]. A CVM was defined as a structural malformation involving intracardiac structures or great arteries. For this analysis, CVMs were organized by mechanistic groups as proposed by Clark [1995]. Because the term "coarctation" can be used broadly, we specified whether there was a descending thoracic juxtaductal aortic shelf seen in typical non-NF coarctation [Beekman, 1995] or a fusiform or segmental hypoplasia of the thoracic or abdominal aorta.

TABLE I. Literature Review of Cardiovascular Malformations in NF1 Patients*

Author	Year	No. of NF1 patients	No. of CVM patients	%	Age	Gender	Comment
Large series:							
Crowe et al.	1956	223	1 ASD	0.4%	37	M	The single patient was clinically diagnosed.
Kaufman et al.	1972	NS					
		(19 families)	6 total:				None had features of Noonan or Watson
			5 PSV		10	M	syndrome.
					53	M	Unclear if leaflets abnormal; annulus definitely small.
					4	M	May also have had thickening of aortic valve leaflets.
					3	F	Brother (1 mo): COA, ASD. No CLS to diagnose NF1.
							Sister (8): Clinically diagnosed VSD. 3 CLS.
							Brother (7): Clinically diagnosed VSD, CLS, MR.
					18	M	
			1 SVAS		6	M	Paternal uncle with NF1 and PVS.
Neiman et al.	1974	78	5 total:	6.4%			
			Family				May represent NF1-Noonan syndrome.
			1 PSV		2 9/12	M	Brother. Also, MR, VPI.
			1 COA, BAV		4½	F	Sister. Also, MR, "Turner phenotype".
			1 PSV		>18	F	Mother. Also, MR, "Turner phenotype".
			Unrelated cases				
			1 VSD		9/12	M	Location of VSD NS.
			1 ASD		6	M	Location of ASD NS, probably secundum.
			(1 CHB)		5	F	Exclude since there is no underlying CVM.
Holt	1978	NS	3 NS	NS	NS	NS	Supplements preceding series of Crowe et al., and Neiman et al.
Carey et al.	1979	131 (60 families)	2 NS	1.5%	NS	NS	
Schorry et al.	1989	78	2 NS	2.6%	NS	NS	
Colley et al.	1996	453	9 PS	2.0%	NS	NS	
		(235 families)	(3 PS)	(0.7%)	NS	NS	(After excluding 4 patients with NF1-Noonan syndrome and 2 patients with Watson syndrome).
Tonsgard et al.	1997	35	3 total:	8.6%			From general NF1 cohort, 70 had features of deletions.
		(406 total)	2 PPS		10, 14	NS	35 (26 families) were studied, 4 had deletions.
			1 VSD, NOS		11	NS	
Small series or individual case reports:							
Zoethout et al.	1964	1	1 ASV	NS	39	M	1 NF patient in a series of 126 patients with AS.
Rosenquist et al.	1970	NS	1 RVOT membrane	NS	9	F	Right ventricular hypertrophy probably secondary to RVOT, not a primary cardiomyopathy.
Pernot et al.	1971	NS	3 PSV	NS	9	F	All 3 had CLS, none had neurofibromas.
					2	F	Unclear if NF1, NF1-Noonan syndrome,
					1	F	Watson. Fourth patient described as having lentiginosis.
Wille et al.	1980						See Table III for description. Unknown if "aortic obstruction" was valvar (ie., a CVM), or subaortic hypertrophy (ie., HCM).
Shimada et al.	1981	1	1 ECD	NS	24	M	
Leppig et al.	1994	5	1 ASD, NOS		5	M	All 5 patients had dysmorphic features, MR, and NF1 gene deletions.
Wu et al.	1995	4	1 PS murmur		16	M	All 4 patients had dysmorphic features, MR, and NF1 gene deletions.
Stoll et al.	1995	1	1 dTGA, TriAtr, PAS, ASD, VSD		birth	F	NF1 not substantiated. CLS, no NFs. Multiple anomalies (microcephaly, dysmorphic face, scoliosis, short digits), pheochromocytoma. Parents first cousins.
Fischberg et al.	1996	1	1 Vascular ring	NS	birth	M	Esophageal compression due to both vascular ring and disseminated neurofibromatosis (including atrial septum and auricular appendages).

*Not included are patients with segmental or fusiform "coarctation" of the abdominal aorta, which is considered part of the NF1 vasculopathy. AS, aortic stenosis; ASD, atrial septal defect; ASsub, sub-aortic stenosis; ASV, aortic stenosis, valvar; BAV, bicuspid aortic valve; CHB, complete heart block; CLS, café au lait spots; COA, coarctation; CVM, cardiovascular malformation; dTGA, dextro-transposition of great arteries; ECD, endocardial cushion defect; F, female; M, male; MR, mental retardation; NF, neurofibromatosis; NS, not specified, not available or not applicable; PAS, pulmonary artery stenosis; PS, pulmonic stenosis; PPS, peripheral pulmonic stenosis; RVOT, right ventricular outflow tract obstruction; TriAtr, tricuspid atresia; VPI, velopharyngeal insufficiency; VSD, ventricular septal defect.

TABLE II. Literature Review of Cardiomyopathy in NF1*

Author	Year	No. of patients and type of CM	Age	Gender	Location	Severity	Comment
Pung and Hirsch	1955	1 "HCM"	4	F	BVH	severe	Hypertrophic ventricles probably due to extrinsic neurofibromas.
Goodwin et al.	1974	1 NS	NS	NS	LV	NS	Mentioned briefly in text, no details.
Gerbaux et al.	1974	1 HCM	13	M	LV	moderate	"Obstructive myocardiopathy". Described as having a Turner phenotype ("male Ullrich") and NF1. Suspect NF1-Noonan syndrome.
Elliot et al.	1976	1 HCM	44	F	LV	moderate	"IHSS"
Benotti et al.	1980	1 RCM	56	M	BVH	NS	
Wille et al.	1980	2 HCM Family					
		father	68	M	LV	severe	Probands had both "aortic and outflow obstruction" and NF1. One daughter had NF1. Seven other relatives had HCM. Likely that pedigree illustrates 2 diseases co-segregating in probands. One proband (at least) extremely dysmorphic.
		son	29	M	LV	NS	
Mercier et al.	1981	2 HCM	(<11)	NS	LV	NS	Briefly mentioned in text.
			(<11)	NS	LV	NS	Age range 7 days–11 yrs.
Sachs et al.	1984	2 HCM	70	M	LV	severe	Post-mortem histology non-specific (without myofiber disarray).
			51	M	LV	severe	
Tillous-Borde et al.	1986	1 HCM	1½	F	BVH	severe	Spontaneous regression at 6 weeks. Maternal diabetes not present.
Hosokawa et al.	1986	1 HCM	71	F	LV	moderate	"IHSS"
Waxman et al.	1986	1 HCM	57	F	LV	NS	"IHSS"
Salvadori et al.	1986	1 HCM	38	M	LV	moderate	HCM, but not IHSS. Localized to infero-apical portion of septum and postero-lateral wall.
Schrader et al.	1986	1 HCM	58	F	LV	severe	Myocardial biopsy showed fiber disproportion and interstitial fibrosis.
Fitzpatrick and Emanuel	1988	2 HCM Family					Seven other family members with NF1 without cardiac disease.
		sister	46	F	LV	severe	
		brother	42	M	LV	severe	No one with HCM alone.

*BVH, biventricular; HCM, hypertrophic cardiomyopathy; IHSS, idiopathic subaortic stenosis; LV, left ventricle; NS, not specified; RCM, restrictive cardiomyopathy.

We recorded information about the method of CVM diagnosis, i.e., whether it was based clinically using auscultation, radiography and electrocardiography, or whether it was confirmed using echocardiography, catheterization, surgery or autopsy. If the NF clinician attributed a murmur to a specific CVM, the patient was said to have a clinically diagnosed CVM. We excluded patients with heart murmurs that were called "functional," however, or if an echocardiogram proved that a suspicious murmur had no anatomic basis. Left superior vena cava to the coronary sinus was also excluded as a normal variant. In addition to CVMs, we tabulated cardiomyopathy, "dysplastic" valve defects (e.g., mitral valve prolapse), intracardiac tumors and electrocardiographic abnormalities.

RESULTS

At the time of this analysis, the NNFFID contained 2550 definite or possible NF1 patients (Table IV). One hundred-twenty patients were submitted for review as having NF1 and a cardiac abnormality; 104 of these were verified as having NF1 according to the NIH diagnostic criteria.

Among the 228 patients who did not meet the NIH criteria for diagnosis of NF1 were 1 patient with LEOPARD syndrome and 15 submitted as having a cardiovascular abnormality. Eleven of these 15 patients were less than 10 years old and had multiple café-au-lait spots but no other features that would establish a diagnosis of NF1 according to the NIH criteria. Five of these 11 young patients had pulmonic stenosis, specified as valvar in one.

Seven of the 104 patients who met the NIH diagnostic criteria for NF1 and were originally submitted as having a cardiovascular anomaly were excluded because they were found not to have a cardiac abnormality or only to have a minor one that could be considered a normal variant (Table IV). The remaining 97 patients with well-established NF1 and a clear diagnosis of cardiovascular disease comprised the cohort that was studied further. Two of these patients had Watson syndrome, and two had the NF1-Noonan phenotype.

Table V lists clinical characteristics of the entire cohort and those with a CVM. Overall, there was a slight male predominance and young age (<10 years). All 4 Watson and NF1-Noonan patients were 6 years old or younger. The diagnosis of most (65%) CVMs was either clinical or not stated. The diagnosis was confirmed by echocardiography alone or with another test in 30%.

Fifty-five (56%) of the 97 verified NF1 patients with

TABLE III. Literature Review of Intracardiac Tumors and Other Cardiac Abnormalities in NF1*

Author	Year	No. of patients	Age	Gender	Comment
Intracardiac Tumors:					
Frankiewicz	1973	1	16	M	Tumor: left atrial neurofibroma.
McAllister & Fenoglio	1978	2	NS	NS	Tumor: neurofibroma. Unclear which 2 of the 3 reported patients with neurofibroma of the pericardium (1) and myocardium (2) had NF1.
Mata et al.	1981	1	27	F	Tumor: myocardial rhabdomyosarcoma.
Takeda et al.	1982	1	NS	NS	Tumor: intrapericardial (histology not stated).
Fischberg et al.	1996	1			Tumor: atrial septum and appendages (in addition to vascular ring, vide supra).
Noubani et al.	1997	1	10	F	Tumor: infiltration of heart from mediastinal neurofibroma. (Also, mitral valve disease secondary to rheumatic fever.)
Miscellaneous:					
Etches and Pickering	1978	1	11	F	MVP clinically, not confirmed.
Halper and Factor	1984	1	38	M	Nonocclusive coronary artery thickening.
Bensaid et al.	1986	1	67	M	MVP by echocardiography.
S. ɔtto di Uccio et al.	198ε	1	52	M	MVP by echocardiography.
Kandarpa et al.	1988	1	30	M	Coronary aneurysm and myocardial infarction.
Uren et al.	1988	1	28	M	Left atrial wall aneurysm.
Nogami et al.	1991	2	52	F	Coronary artery spasm, "myocarditis", apical cardiac sympathetic denervation
			50	F	Right ventricle myofiber disarrary with interstitial fibrosis.
Kaplan and Rosenblatt (Family A, proband),	1985	1	17	M	Aortic dilation, AR, MVP. Patient thought to have NF1-Noonan phenotype.
restudied by Leppig et al. (UWA 169-1)	1997				Later, found to have microdeletion of NF1.
Wu et al.	1997	1	45	M	MVP. Patient is the father of 15-year-old male, both of whom had NF1 deletions.

*AR, aortic regurgitation; MVP, mitral valve prolapse.

cardiovascular disease in this study had a CVM. Patients with CVMs represent 2.3% (54/2322) of the NF1 patients in the NNFFID (Table VI). Excluding 8 patients in whom a CVM was reported as "possible" or inadequately specified, the frequency decreases to 2.0%.

Information is not available on the expected prevalence of CVMs in people without NF1 who represent the same populations and age distributions as our patients. The Baltimore-Washington Infant Study (BWIS) is a recent large population-based study of CVMs in infants (<1 year old) [Ferencz et al., 1993]. Although these data are not strictly comparable to those of the NNFFID, we have included the BWIS population frequencies and distribution of CVMs in Table VI to provide a general frame of reference.

Most (43/54, 80%) of the CVMs in NF1 patients could be classified as "flow defects," in which right or left heart obstruction or simple shunts are postulated to be the result of abnormal embryonic intracardiac hemodynamics [Clark, 1995]. Most (25/43) of the patients with flow defects had pulmonic stenosis, usually specified (or presumed) as valvar. Three of the 4 children with Watson syndrome or NF1-Noonan syndrome had

TABLE IV. Summary of NF1 Patients

Total no. of patients	NIH diagnostic criteria fulfilled: Yes	No
2550	2322	228
Cardiovascular abnormality present	97	
Cardiovascular malformation (Table VI)	54	5
No syndrome	50	
Watson syndrome	2	
NF1-Noonan syndrome	2	
		< 10 years 5 (all pulmonic stenosis)
Other cardiovascular abnormality (Table VII)	27	not studied
Peripheral vascular abnormality (Table VIII)	16	not studied
Cardiovascular malformation present, dysmorphic (reclassified as LEOPARD syndrome)		1
Submitted as having a cardiac abnormality		
Not verified	7	10
Murmur, but echocardiogram was normal	3	
Left superior vena cava	2	
No cardiac diagnosis	2	
		< 10 years 6
		> 10 years 4

TABLE V. Clinical Characteristics of 97 Verified NF1 Patients with Cardiovascular Abnormalities*

	Number of patients	
	Total	CVM
Total	97 (100%)	54 (56%)
Gender		
Female	36 (37%)	23 (43%)
Male	51 (53%)	31 (57%)
Age		
≤10 years	45 (46%)	31 (57%)
11–20 years	24 (25%)	14 (26%)
≥21 years	18 (19%)	9 (17%)
Method used to diagnose the CVM		
Clinical		6 (11%)
Echocardiography		9 (17%)
Echocardiography and another test		7 (13%)
Catheterization		0
Surgery		3 (6%)
Autopsy		0
Not stated		29 (54%)

*CVM, cardiovascular malformation

pulmonic stenosis, and one boy from Children's Hospital in Boston with a pulmonic stenosis murmur has been reported as having a large *NF1* deletion [96–665 in Wu et al., 1995, 1997; Riva et al., 2000].

Left heart obstruction (aortic stenosis, coarctation) was reported in 7 patients. Of 5 patients with "coarctation," however 3 had sufficiently detailed medical records to determine that there was a long fusiform narrowing of the aorta. This differs from the juxtaductal shelf (infolding) usually seen with coarctation in the general population [Beekman, 1995].

In this study, there were also 2 patients with tetralogy of Fallot, a conotruncal CVM (Class I) attributed to altered mesenchymal cell migration. There were no patients with atrioventricular canal defects (Class IV), anomalous pulmonary venous return (Class V), or looping or laterality defects (Class VI). In the Baltimore-Washington Infant Study of newborns [Ferencz et al., 1993], Class II "flow" defects occurred in 70% of patients with CVMs, followed in frequency by Class I "conotruncal" (or outflow) defects (16%), and Class IV "atrioventricular canal" defects (8%). Four hundred-sixty (11%) of the 4308 children with CVMs in the Baltimore-Washington Infant Study had pulmonic stenosis. The proportion of all CVMs that were pulmonic stenosis was 4.3 times (95% confidence interval, 3.2–5.8 times) greater among the NF1 patients than among the patients in the BWIS. The proportion of all CVMs that were coarctation of the aorta was 3.2 times (95% confidence interval, 1.4–7.4 times) greater among the NF1 patients than in the BWIS.

To complete a full description of the NF1 cohort with cardiovascular disease, Table VII lists 27 patients (28% of the 97 reported with cardiovascular abnormalities) with NF1 and other cardiac abnormalities, most of whom had a murmur (unspecified 10, systolic 6). There were no patients in this group from the NNFFID with HCM. Table VIII also lists 16 NF1 patients who had a peripheral vascular abnormality. Four of these patients also had a CVM and are included on Table VI. The observed frequency of peripheral vascular abnormalities probably underestimates the true prevalence because vasculopathy is unlikely to be found in our patients unless they become symptomatic or hypertensive or both.

DISCUSSION

The participants in the NNFFID are a heterogeneous group of NF centers who contribute the data voluntarily. Because most are at tertiary level hospitals, the NF1 patients they see may be more likely to have serious medical problems (such as CVMs) than an unselected group of NF1 patients. On the other hand, very few of the clinics have any particular interest in cardiac disease, as demonstrated in many cases by the poor quality of data recorded about the CVMs. This study is the largest review of cardiac abnormalities in NF1 published to date. The overall frequency of 2% of CVMs among NF1 patients without Watson or NF1-Noonan syndrome in the NNFFID is similar to what was reported by Carey et al. [1979] and Schorry et al. [1989] (Table I). Neither study reported the type of CVM present. Colley et al. [1996] also reported a frequency of 2.0%, but after excluding the patients with Watson (2) or NF1-Noonan (4) syndrome, this figure decreases 0.7%. Perhaps more striking than the overall frequency of CVMs in the Colley et al. [1996] series was that pulmonic stenosis was the sole CVM reported.

The estimate of total CVMs in NF1 obtained from the NNFFID (approximately 20/1000) cannot be directly compared to the population incidence (approximately 5/1000) of children ascertained to age 1 year in the Baltimore-Washington Infant Study [Ferencz et al., 1993]. Data about the frequency of CVMs in older children and young adults in the general population are scant. One small regional study reported a prevalence of 12.5/1000 at age 16 years [Roy et al., 1994]. Our patients range in age from infancy to late adulthood, although most are children (46% younger than 10 years) or young adults who come from specialized NF1 centers throughout the world. Therefore, our patients cannot be assumed to be representative of either all individuals with NF1 or of any particular normal population.

It is likely that some clinically mild CVMs were undetected during the BWIS ascertainment period and recognized after age one year. Nevertheless, the prevalence of CVMs and the relative frequencies of the various defects found in the BWIS are probably the most reliable figures currently available and are similar to those provided in other population-based studies of CVMs in infants and children [Table VIII in Lin et al., 1999]. Although not strictly comparable to the NNFFID data, the BWIS provides a useful general frame of reference for our study.

Cardiovascular malformations in NF1 may have been underestimated in this study if some of the NNFFID patients are not yet old enough to permit a confident diagnosis of NF1 according to the NIH Diagnostic Criteria [DeBella et al., 2000] or if some CVMs were not detected or inadequately documented to permit inclusion in this study. There may have been an overestimate due to referral bias because NF1 patients

TABLE VI. Frequency and Type of Cardiovascular Malformations in 54 NF1 Patient (NNFFID = 2322)*

	Patients with NF1				BWIS	
Clark Class	Confirmed[a]	Not confirmed	No. of patients	(%)	No. of patients	%
Total	19	35	54	(100%)	4308[b]	(100%)
Conotruncal, outflow	1	1	2	(4%)	673	(16%)
Tetralogy of Fallot	1	1	2		297	
Flow	17	26	43	(80%)	3028	(70%)
Right heart, total	10	15	25			
Pulmonic stenosis, total	10	15	25	(46%)	460	(11%)
Valvar	7[c]	2	9			
Peripheral	0	1	1			
NS, presumed valvar	2	10	12			
With VSD	1[d]	1	2			
With ASD	0	1	1			
Left heart, total	5	2	7			
Aortic stenosis, total	1	1	2		74	
Valvar	1	0	1			
NS (presumed valvar)	0	1	1			
Coarctation	4	1	5	(9%)	126	(3%)
Simple shunts, total	2	9	11	(20%)	1955	(43%)
ASD, probably secundum	0	4	4			
VSD, total	1	5	6			
NS	0	4	4			
With SVPS + DC RV	1	0	1			
With ASD	0	1	1			
PDA	1	0	1			
MVP, mitral regurgitation	1[e]	0	1		NS	
Possible CVM, NS, or inadequately specified	0	8	8	(15%)	NS	

*[Clark, 1995; Ferencz et al., 1997].
[a]Confirmed by two-dimensional echocardiography, catheterization, or surgical observation.
[b]Excludes cardiomyopathy (82 patients).
[c]Includes Watson syndrome (1), NF1-Noonan syndrome (1).
[d]Includes Watson syndrome (1).
[e]Includes NF1-Noonan syndrome (1).
ARSCA, aberrant right subclavian artery; ASD, atrial septal defect; BWIS, Baltimore-Washington Infant Study; DC RV, double chambered right ventricle; MVP, mitral valve prolapse; NS, not specified; PDA, patent ductus arteriosus; PS, pulmonic stenosis; SVPS, supra-valvar pulmonic stenosis; VSD, ventricular septal defect.

with CVMs were more likely to be seen by specialists who would recognize NF1. Finally, because a large proportion of the reported CVMs in this series were diagnosed clinically (28%) or not stated (41%), we cannot be certain that a CVM was present. Little information was available about karyotype or NF1 microdeletions. None of our NF1 patients with CVM had DNA studies or microdeletion studies for other loci (e.g., 22q11).

The study's most striking finding is the predominance of pulmonic valve stenosis among NF1 patients. The proportion of all CVMs that were pulmonic stenosis among the NF1 patients was almost 6 times greater than among the patients in the BWIS. This preponderance of pulmonic stenosis, usually valvar, among NF1 patients is consistent with the clinical series and case reports that have previously been published (Table I) and provides strong evidence that *NF1* gene mutations predispose to the development of this particular CVM. The possibility of pulmonic stenosis should be considered in any NF1 patient with a systolic murmur. Similarly, the diagnosis of NF1 should be considered in any child with pulmonic stenosis who has multiple café au lait spots.

TABLE VII. Other Cardiovascular Abnormalities in 27 Patients with NF1

Abnormality	No. of patients
Cardiomyopathy	0
Murmur, no echocardiogram (CVM has not been ruled out)	16
Systolic	6
Not specified	10
Mitral valve prolapse +/- mitral regurgitation	5
Tumor	
possible myxoma (possible cardiac neurofibroma)	1
Electrocardiogram abnormality	
first degree heart block	2
changes of left ventricular myocardium	3

The apparently increased proportion of NF1 patients with CVMs who have coarctation must be viewed with caution. In 3/5 NF1 patients, the coarctation was a fusiform narrowing of the descending thoracic aorta, that differs from the typical juxtaductal shelf seen in the general population [Beekman, 1995]. This observation and the apparent association between the occurrence of renal artery stenosis and coarctation in NF1 patients suggests that coarctation may often be a manifestation of NF vasculopathy rather than a true CVM. We did not observe peripheral vascular anomalies in any NF1 patients with coarctation of the aorta in this study (Table VIII).

An equally important finding is the low frequency of more complex CVMs such as conotruncal, atrioventric-

TABLE VIII. Peripheral Vascular Abnormalities in 16 Patients with NF1

Vascular abnormality	No. of patients	4 additional patients who had a CVM (as listed on Table VI) and a peripheral vascular abnormality
Renal artery stenosis	9	1 pulmonic stenosis, valvar + renal and femoral artery stenosis 1 pulmonic stenosis, not specified + renal artery stenosis
Subclavian artery stenosis	1	
Middle cerebral artery stenosis	1	
Moya moya disease	2	
Arteriovenous malformations left temporal lobe	1	
Aneurysm right subclavian artery	1	1 multiple CHD, not specified + splenic artery aneurysm
"Bruits", location not specified	1	1 pulmonic stenosis, not specified + unspecified stenosis

ular, laterality and looping defects. The paucity of conotruncal CVMs in patients with NF1 is in striking contrast to the *Nf1* "knockout" mouse homozygous mutant embryos [Brannan et al., 1994; Jacks et al., 1994]. These embryos die with double outlet right ventricle, a CVM attributed to abnormal migration of ectomesenchymal cells [Clark, 1995] derived from the cranial neural crest [Kirby et al., 1983]. Furthermore, these mice embryos have enlarged and abnormal endocardial cushions [Lakkis and Epstein, 1998] that may obstruct forward blood flow in some cases. This anomaly suggests that neurofibromin is required for normal cardiac valve development.

Complex developmental mechanisms in addition to neural crest migration play a role in cardiac development in NF1. The rarity of complex CVMs supports the notion that NF1 is not a multiple malformation syndrome and may better be viewed as a multiple dysplasia syndrome [Carey and Viskochil, 1999].

Cardiac hypertrophy has been reported in a small number of patients with NF1 (Table III). In 14 of the 18 reported patients, this was probably primary HCM (usually left ventricular, idiopathic hypertrophic subaortic stenosis), although extrinsic compression by a neurofibroma caused biventricular hypertrophy in at least one girl [Pung and Hirsch, 1995]. In 2 patients, HCM and NF1 were probably segregating as independent autosomal dominant traits [Wille et al., 1980]. One boy may have had the NF1-Noonan syndrome [Gerbaux et al., 1974]. Two patients had either postmortem examination [Sachs et al., 1984] or biopsy [Schrader et al., 1986] with myocardial examination showing non-specific changes and fiber disproportion with fibrosis, respectively.

No patient in the NNFFID had HCM (Table II). Although this may reflect a genuine lack of association, it may just be due to small sample size and insufficient statistical power to detect an association. The reported prevalence of HCM varies with age. In newborns followed to age one year in the Baltimore-Washington Infant Study, the prevalence was 0.4 per 10,000 [Ferencz et al., 1993]. This is similar to a retrospective study from Finland that reported 0.3 per 10,000 [Arola et al., 1997]. In contrast, an oft quoted frequency of HCM among healthy adults (23–35 years) is 20 per 10,000 (1 per 500) [Maron et al., 1995]. Power calculations indicate that we are unlikely to have detected even a 5-fold increase in the prevalence of HCM in this study.

It remains unclear whether HCM represents a rare manifestation of the NF1 mutation or a chance occurrence. Of patients in the literature (Table II), none has had molecular analysis for either the *NF1* or common HCM mutations [Coonar and McKenna, 1997]. Because neurofibromin is expressed in the myocardium of the developing heart and the cardiac muscle becomes thinned in homozygous *Nf1* mutant mice [Brannan et al., 1994; Jacks et al., 1994; Lakkis and Epstein, 1998], there is biologic plausibility that myocardial disease may occur in NF1.

There is no firm genotype-phenotype correlation with particular *NF1* mutations. NF1 patients with deletions of the entire gene frequently have dysmorphic features and intellectual limitations [Leppig et al., 1994; Wu et al., 1995, 1997; Tonsgard et al., 1997; Riva et al., 2000], but these clinical features are not obligatory [Rasmussen et al., 1998]. A CVM was noted in 3 reported patients with large deletions [Leppig et al, 1994; Wu et al., 1995, 1997; Patient 3 in Tonsgard et al., 1997] (Table I). Because the NIH criteria remain the "gold standard" for diagnosis, molecular analysis for *NF1* mutations was not routinely done for patients in the NNFFID.

At present, all individuals with NF1 should have careful cardiac auscultation and blood pressure monitoring as part of every NF-related examination. The value of routine echocardiography for all NF1 patients has not been established. There is little disagreement that a child with NF1 and a murmur, especially if there are associated dysmorphic features, needs a cardiology evaluation. This will likely include an echocardiogram.

To obtain more accurate information about CVMs in NF1, a multi-center study is needed involving geneticists and cardiologists to ensure accurate diagnosis of both NF1 and cardiac abnormalities. Cross-sectional echocardiography could be used to evaluate patients in various age groups. The natural history of pulmonic stenosis in NF1 should be studied to compare its progression (or regression) with the general population. Pulmonic stenosis in NF1 seems to be generally mild and not a cause of serious morbidity, but this clinical impression requires confirmation. To pursue an investigation of HCM in NF1, echocardiography could also be used to monitor for obvious hypertrophy and subtler increased cardiac mass.

CONCLUSIONS

Among patients entered into the NNFFID, there was a higher than expected frequency of pulmonic stenosis and aortic coarctation. Appropriate cardiac examination and blood pressure monitoring should be part of the routine care of individuals with NF1, and those found to have abnormalities should be referred for cardiovascular assessment and treatment.

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REFERENCES

Arola A, Jokinen E, Ruuskanen O, Saraste M, Pesonen E, Kuusela AL, Tikanoja T, Paavilainen T, Simell O. 1997. Epidemiology of idiopathic cardiomyopathies in children and adolescents. A nationwide study in Finland. Am J Epidemiol 146:385–393.

Allanson JE, Upadhaya M, Watson GH, Partington M, MacKenzie A, Lahey D, MacLeod H, Sarfarazi M, Broadhead W, Harper PS, Huson SM. 1991. Watson syndrome: is it a subtype of type 1 neurofibromatosis? J Med Genet 28:752–756.

Beekman RH. 1995. Coarctation of the aorta. In: Emmanouilides GC, Allen HD, Riemenschneider TA, Gutgesell HP, editors. Moss and Adams' heart disease in infants, children, and adolescents including the fetus and young adult. Baltimore: Williams and Wilkins. p 1111.

Benotti JR, Grossman W, Cohn PF. 1980. Clinical profile of restrictive cardiomyopathy. Circulation 61:1206–1212.

Bensaid J, Gueret P, Virot P, Vernoux J, Lacroix P, Thiry M. 1986. Maladie de Recklinghausen et prolapsus valvulaire mitral. Presse Med 15:1424.

Brannan CI, Perkins AS, Vogel KS, Ratner N, Nordlund ML, Ried SW, Buchberg AM, Jenkins NA, Parada LF, Copeland NG. 1994. Targeted disruption of the neurofibromatosis type-1 gene leads to developmental abnormalities in heart and various neural crest-derived tissues. Genes Dev 8:1019–1029.

Carey JC, Laub JM, Hall BD. 1979. Penetrance and variability in neurofibromatosis: a genetic study of 60 families. Birth Defects Orig Artic Ser 14:271–281.

Carey JC, Viskochil DH. 1999. Neurofibromatosis, type 1: a model condition for the study of the molecular basis of variable expression in human disorders. Am J Med Genet (Semin Med Genet) 89:7–13.

Clark EB. 1995. Epidemiology of congenital cardiovascular malformations. In: Emmanouilides GC, Allen HD, Riemenschneider TA, Gutgesell HP, editors. Moss and Adams' heart disease in infants, children, and adolescents including the fetus and young adult. Baltimore: Williams and Wilkins. p 60–70.

Colley A, Donnai D, Evans DGR. 1996. Neurofibromatosis/Noonan phenotype: a variable feature of type 1 neurofibromatosis. Clin Genet 49:59–64.

Coonar AS, McKenna WJ. 1997. Molecular genetics of familial cardiomyopathies. Adv Genet 35:285–324.

Crowe FW, Schull WJ, Nee JV. 1956. A clinical, pathological, and genetic study of multiple neurofibromatosis. Springfield, IL: Charles C. Thomas. p 100.

DeBella K, Szudek J, Friedman JM. 2000. Use of the National Institutes of Health criteria for diagnosis of neurofibromatosis 1 in children. Pediatrics 105:608–614.

Elliott CM, Tajik AJ, Giuliani ER, Gordon H. 1976. Idiopathic hypertrophic subaortic stenosis associated with cutaneous neurofibromatosis: report of a case. Am Ht J 92:368–372.

Etches PC, Pickering D. 1978. Apical systolic click and murmur associated with neurofibromatosis. J Med Genet 15:401–403.

Ferencz C, Loffredo CA, Rubin JD, Magee CA. 1993. Epidemiology of congenital heart disease. The Baltimore-Washington Infant Study 1981–89. Mount Kisco, NY: Futura Publishing Company, Inc.

Ferencz C, Loffredo CA, Correa-Villasenor A, Wilson PD. 1997. Genetic and environmental risk factors of major cardiovascular malformations. The Baltimore-Washington Infant Study 1981–89. Mount Kisco, NY: Futura Publishing Company, Inc.

Fischberg C, Cotting J, Hack I, Laurini RN, Payot M. 1996. Double compression oesotracheale fatale vasculaire et neurofibromateuse. Arch Pediatr 3:1252–1257.

Fitzpatrick AP, Emanuel RW. 1988. Familial neurofibromatosis and hypertrophic cardiomyopathy. Br Heart J 60:247–251.

Frankiewicz L. 1973. Rare case of v. Recklinghausen disease with a tumor in the left atrium. Pol-Tyg-Lek 28:411–412.

Friedman JM, Gutmann DH, MacCollin M, Riccardi VM, eds. 1999. Neurofibromatosis: phenotype, natural history, and pathogenesis, 3rd ed. Baltimore: Johns Hopkins University Press.

Friedman JM. 1999. Vascular abnormalities in NF1. In: Friedman JM, Guttman DH, Mac Collin M, Riccardi V, eds. Neurofibromatosis: phenotype, natural history, and pathogenesis. 3rd ed. Baltimore: Johns Hopkins University Press.

Friedman JM, Birch PH, and the NNFF International Database Participants. 1997. Cardiovascular malformations in neurofibromatosis type 1 (NF1). Am J Hum Genet 61:A98.

Friedman JM, Birch PH, Greene C, and the NNFF International Database Participants. 1993. National Neurofibromatosis Foundation International Database. Am J Med Genet 45:88–91.

Gerbaux A, Belfante M, Hiltgen M. 1974. Myocardiopathie obstructive, syndrome de Turner-Ullrich et maladie de Von Recklinghausen. Ann Med Int 125:641–650.

Goodwin JF.1974. Prospects and predictions for the cardiomyopathies. Circulation 50:210–219.

Halper J, Factor S. 1984. Coronary lesions in neurofibromatosis associated with vasospasm and myocardial infarction. Am Heart J 108:420–423.

Holt JF. 1978. Neurofibromatosis in children. Am J Roentgenol 130:615–639.

Hosokawa T, Iwabuchi K, Ohe Y, Sato H, Konno H, Haga S, Sakakibara N. 1986. General anesthesia for a patient with IHSS (idiopathic hypertrophic subaortic stenosis) and von Recklinghausen disease. Masui 35: 450–454.

Huson SM, Hughes RAC. 1994. The neurofibromatoses: a pathogenic and clinical overview. London: Chapman and Hall.

Jacks T, Shih TS, Schmitt EM, Bronson RT, Bernards A, Weinberg RA. 1994. Tumour predisposition in mice heterozygous for a targeted mutation in Nf1. Nat Genet 7:353–361.

Jamieson CR, van der Burgt I, Brady AF, van Reen M, Elsawi MM, Hol F, Jeffery S, Patton MA, Mariman E. 1994. Mapping a gene for Noonan syndrome to the long arm of chromosome 12. Nat Genet 8:357–360.

Kirby ML, Gale TF, Stewart DE. 1983. Neural crest cells contribute to aorticopulmonary septation. Science 220:1059–1061.

Kandarpa K, Stoll JF, Reiss C, Rutherford JD, Cohn LM. 1988. A case of neurofibromatosis associated with a coronary artery aneurysm and myocardial infarction. Cardiovasc Intervent Radiol 11:143–145.

Kaplan P, Rosenblatt B. 1985. A distinctive facial appearance in neurofibromatosis von Recklinghausen. Am J Med Genet 21:463–470.

Kaufman RL, Hartmann A, McAlister WH. 1972. Family studies in congenital heart disease IV: congenital heart disease associated with neurofibromatosis. Birth Defects Orig Artic Ser VIII:92–95.

Kayes LM, Burke W, Riccardi VM, Bennett R, Ehrlich P, Rubenstein A, Stephens K. 1994. Deletions spanning the neurofibromatosis I gene: identification and phenotype of five patients. Am J Hum Genet 54:424–436.

Lakkis MM, Epstein JA. 1998. Neurofibromin modulation of ras activity is required for normal endocardial-mesenchymal transformation in the developing heart. Development 125:4359–4367.

Leppig KA, Kaplan P, Viskochil D, Weaver M, Ortenberg J, Stephens K. 1997. Familial neurofibromatosis 1 microdeletions: cosegregation with distinct facial phenotype and early onset of cutaneous neurofibromata. Am J Med Genet 73:197–204.

Lin AE, Garver KL. 1988. Cardiac abnormalities in neurofibromatosis. Neurofibromatosis 1:146–151.

Lin AE, Pexieder T. 1994. Need for greater precision in reporting cardiovascular malformations. Am J Med Genet 51:84–85.

Lin AE, Herring AH, Scharenberg KA, Westgate M-N, Lacro RV, Al-Jufan M, Ryan L, Holmes LB. 1999. Cardiovascular malformations: Changes in prevalence and birth status, 1982–90. Am J Med Genet 82:102–110.

Mata M, Wharton M, Geisinger K, Pugh JE. 1981. Myocardial rhabdomyosarcoma in multiple neurofibromatosis. Neurology 31:1549–1662.

McAllister HA, Fenoglio JJ. 1978. Tumors of the cardiovascular system. Atlas of tumor pathology, second series. Fascicle 15. Washington, DC: Armed Forces Institute of Pathology. p 70–71.

Marino B, Digilio MC, Toscano A, Giannotti A, Dallapiccola B. 1999. Congenital heart diseases in children with Noonan syndrome: an expanded cardiac spectrum with high prevalence of atrioventricular canal. J Pediatr 135:703–706.

Maron BJ, Gardin JM, Flack JM, Gidding SS, Kurosaki TT, Bild DE. 1995. Prevalence of hypertrophic cardiomyopathy in a general population as young adults: echocardiographic analysis of 4111 subjects in the CARDIA study. Circulation 92:785–789.

Mercier JN, ZiZi A, Fermont L. 1981. Myocardiopathies hypertrophiques obstructives de l'enfant. Ann Pediatr 28:359–365.

Neiman HL, Mena E, Holt JF, Stern AM, Perry B. 1974. Neurofibromatosis and congenital heart disease. Am J Roentgenol 122:146–149.

NIH Consensus Development Conference. 1988. Neurofibromatosis. Arch Neurol 45:575–578.

Nogami A, Hiroe M, Marumo F. 1991. Regional sympathetic denervation in von Recklinghausen disease with coronary spasm and myocarditis. Int J Cardiol 32:397–400.

Noubani H, Poon E, Cooper RS, Kahn E, Kazadevich M, Parnell VA. 1997. Letter: neurofibromatosis with cardiac involvement. Pediatr Cardiol 18:156–158.

Pernot C, Deschamps JP, Didier F. 1971. Stenoses de l'artere pulmonaire taches cutanees pigmentaires et anomalies du squelette. Arch Franc Ped 28:593–603.

Pung S, Hirsch EF. 1955. Plexiform neurofibromatosis of the heart and neck. Arch Pathol 59:341–346.

Rasmussen SA, Colman SD, Ho VT, Abernathy CR, Arn PH, Weiss L, Schwartz C, Saul RA, Wallace MR. 1998. Constitutional and mosaic large NF1 gene deletions in neurofibromatosis type I. J Med Genet 35:468–471.

Riccardi VM. 1992. Neurofibromatosis: phenotype, natural history, and pathogenesis, 2nd ed. Baltimore: The Johns Hopkins University Press.

Riva P, Corrado L, Natacci F, Castorina P, Wu BL, Schneider GH, Clementi M, Tenconi R, Korf BR, Larizza L. 2000. *NF1* microdeletion syndrome: refined FISH characterization of sporadic and familial deletions with locus-specific probes. Am J Hum Genet 66:100–109.

Rosenquist GC, Krovetz LJ, Haller JA, Simon AL, Bannayan GA. 1970. Acquired right ventricular outflow obstruction in a child with neurofibromatosis. Am Heart J 79:103–108.

Roy DL, McIntyre L, Human DG, Nanton MA, Sherman GJ, Allen LM, Finley JP. 1994. Trends in the prevalence of congenital heart disease: Comprehensive observations over a 24-year period in a defined region of Canada. Can J Cardiol 10:821–826.

Rubenstein A, Korf B. 1990. Neurofibromatosis: a handbook for patients, families and health care professionals. New York: Thieme.

Sachs RN, Buschauer-Bonnet C, Kemeny JL, Amouroux J, Lanfranchi J. 1984. Myocardiopathie hypertrophique et maladie de von Recklinghausen. Rev Med Interne 5:154–156.

Salvadori A, Bigi R, Corradetti C, Occhi G, Partesana N, Zatta G, Tarolo GL. 1986. Malattia di Recklinghausen e cardiomiopatia ipertrophica. Descrizione di un caso con ipertrofia distrettuale. Min Cardio 34:771–773.

Schorry EK, Stowens DW, Crawford AH, Stowens PA, Schwartz WR, Dignan PSJ. 1989. Summary of patient data from a multidisciplinary neurofibromatosis clinic. Neurofibromatosis 2:129–134.

Schrader R, Kunkel B, Schneider M, Kalatenbach M. 1986. Hypertrophische kardiomyopathie bei einer patientin mit neurofibromatose von Recklinghausen. Med Klin 81:264–267.

Scotto di Uccio V, Petrillo C, Chiosso M, Tommasis L. 1988. Prolasso mitralico e mallattia di Recklinghausen. Descrizione di un caso. Min Cardio 36:331–333.

Sharland M, Burch M, McKenna WM, Paton MA. 1992. A clinical study of Noonan syndrome. Arch Dis Child 67:178–183.

Shen MH, Harper PS, Upadhyaya M. 1996. Molecular genetics of neurofibromatosis type 1 (NF1). J Med Genet 33:2–17.

Shimada E, Asano H, Kurasawa T, Yamane Y. 1981. Neurofibromatosis associated with endocardial cushion defects—a case report. Nippon Rinsho 39:469–472.

Stern HJ, Saal HM, Lee JS, Fain PR, Goldgar DE, Rosenbaum KN, Barker DF. 1995. Clinical variability of type 1 neurofibromatosis: is there a neurofibromatosis-Noonan syndrome? J Med Genet 29:184–187.

Stoll C, Alembik Y, Dott B. 1995. Complex congenital heart disease, microcephaly, pheochromocytoma and neurofibromatosis type 1 in a girl born from consanguineous parents. Genet Couns 6:217–220.

Takeda H, Minagawa Y, Soki H, Watanabe F, Takahashi J, Matsukura H, Yasuda Y, Yokota A, Ohta S, Kawakami T, Tanabe T. 1982. Surgery of intrapericardial tumor associated with von Recklinghausen disease: case report. Kyobu Geka 35:724–728.

Tassabehji M, Strachan T, Sharland M, Colley A, Donnai D, Harris R, Thakker N. 1993. Tandem duplication within a neurofibromatosis type 1 (NF1) gene exon in a family with features of Watson syndrome and Noonan syndrome. Am J Hum Genet 53:90–95.

Tonsgard JH, Yelavarthi KK, Cushner S, Short MP, Lindgren V. 1997. Do NF1 gene deletions result in a characteristic phenotype? Am J Med Genet 73:80–86.

Tillous-Borde I, Challier P, Fontaine JL. 1986. Myocardiopathie hypertrophique regressive chez un nourrisson atteint d'une neurofibromatose de Recklinghausen. Arch Fr Pediatr 43:197–200.

Uren N, Been M, Guzman F. 1988. Congenital left atrial wall aneurysm in a patient with neurofibromatosis. Br Heart J 59:391–394.

Watson GH. 1967. Pulmonary stenosis, cafe-au-lait spots, and dull intelligence. Arch Dis Child 42:303–307.

Waxman BP, Buzzard AJ, Cox J, Stephens MJ. 1986. Gastric and intestinal bleeding in multiple neurofibromatosis with cardiomyopathy. Aust N Z J Surg 56:171–173.

Wille LE, Forre I, Steffensen RW. 1980. A familial syndrome with Von Recklinghausen neurofibromatosis, gammopathy and aorta outflow obstruction. Acta Med Scand 207:297–304.

Wu B-L, Austin MA, Schneider GH, Boles RG, Korf BR. 1995. Deletion of the entire NF1 gene detected by FISH: four deletion patients associated with severe manifestations. Am J Med Genet 59:528–535.

Wu B-L, Schneider GH, Korf BR. 1997. Deletion of the entire NF1 gene causing distinct manifestations in a family. Am J Med Genet 69:98–101.

Zoethout HE, Bonham Carter RE, Carter CO. 1964. A family study of aortic stenosis. J Med Genet 1:2–9.

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NF1 Gene and Neurofibromatosis 1

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Neurofibromatosis 1 (NF1), also known as von Recklinghausen disease, is an autosomal dominant condition caused by mutations of the *NF1* gene, which is located at chromosome 17q11.2. NF1 is believed to be completely penetrant, but substantial variability in expression of features occurs. Diagnosis of NF1 is based on established clinical criteria. The presentation of many of the clinical features is age dependent. The average life expectancy of patients with NF1 is probably reduced by 10–15 years, and malignancy is the most common cause of death. The prevalence of clinically diagnosed NF1 ranges from 1/2,000 to 1/5,000 in most population-based studies. A wide variety of *NF1* mutations has been found in patients with NF1, but no frequently recurring mutation has been identified. Most studies have not found an obvious relation between particular *NF1* mutations and the resulting clinical manifestations. The variability of the NF1 phenotype, even in individuals with the same *NF1* gene mutation, suggests that other factors are involved in determining the clinical manifestations, but the nature of these factors has not yet been determined. Laboratory testing for *NF1* mutations is difficult. A protein truncation test is commercially available, but its sensitivity, specificity, and predictive value have not been established. No general, population-based molecular studies of *NF1* mutations have been performed. At this time, it appears that the benefits of population-based screening for clinical features of NF1 would not outweigh the costs of screening. *Am J Epidemiol* 2000;151:33–40.

neurofibromatosis; neurofibromatosis 1

GENE

The neurofibromatosis 1 (*NF1*) gene is located at chromosome 17q11.2. *NF1* and its protein product, neurofibromin, were characterized in 1990 (1, 2). The gene is large, spanning 350 kilobases of genomic DNA, and contains 60 exons (3). Neurofibromin belongs to a family of proteins that serve as negative regulators of the *ras* oncogene (4). Neurofibromin is believed to act as a tumor suppressor, but the protein has other functions as well. The proposed tumor suppressor function is supported by the findings of somatic "second hit" mutations of the *NF1* gene in benign and malignant tumors from NF1 patients (5, 6).

NF1 is an autosomal dominant condition with virtually 100 percent penetrance by adulthood (7). About 50 percent of NF1 cases result from new mutations. Germline mosaicism has been observed (8) and must be considered when counseling unaffected parents of cases with new mutations. The *NF1* mutation rate is among the highest observed in humans, with estimates ranging from about 1/7,800 to 1/23,000 gametes (7, 9). About 90 percent of new mutations occur on the paternally derived chromosome (10, 11). The exception is large deletions, which are usually of maternal origin (12, 13).

GENE VARIANTS

As of February 1999, the *NF1* Genetic Analysis Consortium documented more than 240 different constitutional *NF1* mutations in its database (http://www.nf.org/nf1gene/). Table 1 summarizes the types of mutations identified thus far. The majority of mutations lead to a truncated protein product; only about 10 percent involve amino acid substitutions, and fewer than 2 percent are 3′ untranslated region mutations. However, it should be noted that the types of mutations identified are largely dependent on the techniques used for mutation detection. This may result in an overrepresentation of mutation types that are more easily identified (e.g., large gene deletions) and an underrepresentation of those that may be more difficult to identify (e.g., mutations in the 3′ untranslated region). None of the methods used for *NF1* mutation detection are capable of identifying all mutation types.

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Abbreviations: NF1, neurofibromatosis 1; NF2, neurofibromatosis 2.

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TABLE 1. Summary of *NF1* mutation types*

Type of mutation	No. of cases
Chromosome abnormality	4
Deletion of entire gene	18
Multi-exon deletion	38
Small deletion	55
Large insertion	3
Small insertion	27
Stop mutation	43
Amino acid substitution	29
Intron mutation	25
3′ untranslated region mutation	4
Total	246

* Reported to the NF1 Genetic Analysis Consortium (http://www.nf.org/nf1gene/) as of February 1999.

Mutations have been identified throughout the gene. While some recur in different families, no true "hotspots" have been found in *NF1*. The most frequently recurring alteration is a nonsense mutation in exon 31 (R1947X) that accounts for 1–2 percent of the *NF1* mutations identified (14).

At this time, no information is available on the frequency of different mutations in different populations and ethnic groups.

DISEASES

Clinical features of NF1

Neurofibromatosis 1 (NF1), also known as von Recklinghausen disease, is the condition most commonly associated with *NF1* gene mutations. Early discussions of NF1 referred to the condition as "neurofibromatosis" and included cases of the much less frequent condition, neurofibromatosis 2 (NF2). However, these conditions are both clinically and genetically distinct. The most characteristic lesions of NF2 are bilateral schwannomas on the vestibular portion of the eighth cranial nerve; such tumors are rarely seen in NF1 patients. NF2 results from mutations in the *NF2* gene on chromosome 22.

Despite advances in understanding of the molecular genetics of NF1, its diagnosis remains a clinical one, based on diagnostic criteria established by a National Institutes of Health consensus conference (15, 16). A diagnosis of NF1 by these criteria requires the presence of two or more of the following: 1) six or more café-au-lait macules more than 5 mm in greatest diameter in prepubertal individuals and more than 15 mm in greatest diameter after puberty; 2) two or more neurofibromas of any type or one plexiform neurofibroma; 3) freckling in the axillary or inguinal regions; 4) an optic pathway tumor; 5) two or more Lisch nodules (iris hamartomas); 6) a distinctive osseous lesion, such as sphenoid wing dysplasia or thinning of the cortex of long bones (with or without pseudarthrosis); or 7) a first-degree relative (parent, sibling, or child) with NF1 diagnosed by the above criteria.

Some of these features, including café-au-lait spots, freckling in non-sun-exposed areas, and iris Lisch nodules, are not of clinical significance beyond their usefulness in making a diagnosis of NF1. Benign cutaneous and subcutaneous neurofibromas are present in nearly all patients with NF1 by adulthood, and their number in an individual varies widely from only a few to hundreds or more. While these lesions are primarily of cosmetic significance, they may be disfiguring and result in significant psychologic distress. In contrast, about 15 percent of individuals with NF1 have plexiform neurofibromas (17). These tumors may extend into contiguous tissues, causing serious functional impairment and even death and appear to be the site of malignant peripheral nerve sheath tumor development. Optic pathway tumors are observed in about 20 percent of the children with NF1, but most such tumors do not cause ophthalmologic or other symptoms (18). Bony changes, such as pseudarthrosis, appear to occur in about 5 percent of the cases (17). Often these changes are benign; however, some patients are severely affected, with long-bone bowing leading to fracture and, in some cases, requiring amputation (19).

Several other features are often associated with NF1, including macrocephaly, scoliosis, short stature, hypertension, and high-T2-signal-intensity lesions on magnetic resonance imaging of the brain (16). Most individuals with NF1 have normal intelligence, but 30–60 percent have learning disabilities (20).

Individuals with NF1 also appear to be at increased risk for malignancy, but the magnitude of this is difficult to estimate, given the paucity of epidemiologic studies. In an investigation of a Danish cohort of 212 NF1 patients followed for 42 years, a relative risk of 4.0 (95 percent confidence interval: 2.8, 5.6) was observed for malignant neoplasms or benign central nervous system tumors among probands. Since the probands had been identified initially through hospitals and might represent a bias toward more severely affected cases, the relative risk was also determined for affected relatives; this risk was 1.5 (95 percent confidence interval: 0.9, 2.4). The risk was greater for females than for males (21).

Certain types of cancers occur more frequently in individuals with NF1. Malignant peripheral nerve sheath tumors, often referred to as neurofibrosarcomas, are the most common malignancy occurring with increased frequency in NF1. These aggressive tumors are relatively resistant to therapy and are often lethal

(22). Central nervous system tumors, including optic pathway tumors, other astrocytomas, ependymomas, medulloblastomas, and others, also occur more frequently in NF1 patients (23). In addition, individuals with NF1 have an increased risk for myeloid leukemias, with over a 200-fold relative risk for chronic myelomonocytic leukemia (24). The increased risk for malignancies in NF1 is compatible with the finding that the NF1 protein serves as a down-regulator of the *ras* oncogene (4). An increased risk for malignancy could be predicted to result from inactivation of this tumor suppressor function through *NF1* mutation.

The presentation of most NF1 features is age dependent. Café-au-lait spots may be present at birth and increase in number in early childhood. Skinfold freckling is most often observed next. Neurofibromas frequently first appear or increase in number between ages 10 and 20 years. Lisch nodules of the iris are often not present in childhood but are seen in nearly all adults with NF1 (17).

Prevalence of NF1

For several reasons, NF1 is a difficult condition for which to determine an accurate prevalence number. First, the wide variability in expression means that mild cases may escape ascertainment in studies dependent on an affected individual coming to medical attention. Second, the age-dependent presentation of most NF1 features means that examination of young children may miss cases that are truly affected with the condition. Third, the increased mortality seen in individuals with NF1 (see Mortality of NF1, below) reduces the prevalence in later adulthood. Prevalence studies are summarized in table 2 and suggest that NF1 is one of the most common autosomal dominant conditions. The prevalence does not appear to differ by gender. The wide variation in prevalence estimates may reflect differences in diagnostic criteria and methods of case ascertainment of the studies; however, the variation may also represent true differences between populations, perhaps due to a founder effect (particularly in smaller populations) or other factors. One study (25) demonstrated differences in NF1 prevalence among various ethnic groups, with a higher prevalence in individuals of North African and Asian origins (1/522 and 1/1,052, respectively) and a lower frequency among individuals of European and North American backgrounds (1/1,562). These differences were statistically significant, and case ascertainment in this study was based on a mandatory physical

TABLE 2. Studies of the prevalence of neurofibromatosis 1

Study site	No. screened	Ethnic origin of population studied	Method of ascertainment	Age of cases ascertained	Estimated prevalence	Reference
Michigan	252,092	Residents of Michigan	Surveys of general hospital admissions and state institutions for the mentally retarded and "epileptic" (estimate extrapolated from these populations)	All ages	1/2,500–1/3,300*	55
USSR	94,000	Primarily "Russian"	Screening examination for 6-café-au-lait spots as part of evaluation for military duty; detailed examination for those initially identified	16 years	1/7,800†	56
Sweden	440,082	Residents of Göteberg, Sweden	Medical record review, letters to medical institutions and physicians, assessment of family members of affected cases	20 years and older	1/4,600	57
Southeast Wales	668,100	Residents of southeast Wales	Medical record review, letters to physicians, assessment of family members of affected cases	All ages	1/4,150‡	7
New Zealand	113,700	British descent with "substantial Scots component"	Medical record review, letters to physicians, assessment of family members of affected cases	All ages	1/2,190	58
Italy	2,375,304	Northeast Italy	Cases from genetics service and from computerized hospital data	All ages	1/6,711	59
Israel	374,440	Primarily from Europe, North America, Asia, North Africa, and Israel	Physical examination as part of evaluation of fitness for military duty	17 years	1/960	25
Finland	732,000	Residents of northern Finland	Medical record review	All ages	1/3,716	22

* Estimated incidence at birth.

† Assumes that about three quarters of the cases of NF1 would be ascertained through mass medical examination for at least six café-au-lait spots.

‡ Corrected estimate based on possible "missed," mildly affected cases, especially in children.

examination for fitness for military service, suggesting that referral bias was not responsible for the observed differences. The question of the true prevalence of NF1 and whether it differs significantly between populations will require further study.

Mortality of NF1

The best available mortality data are from a population-based study of NF1 patients living in Göteberg, Sweden (26). Adults (age 20 years and older) with NF1 were ascertained through multiple medical specialities. The average age at the time of ascertainment was 43.6 ± 15.4 years for the 70 patients followed. Cases were followed for 12 years. Over this time period, 22 of the 70 NF1 patients died; 5.1 deaths were expected on the basis of the general Swedish population. Of these 22 deaths, 13 were women and nine were men, with 1.7 and 3.4 deaths expected in the populations, respectively, leading the authors to suggest that women may be affected more than men. The study showed a significantly reduced life expectancy in patients with NF1 ($p < 0.001$), with a mean age at death of NF1 patients of 61.6 years compared with a life expectancy in the general population of 75 years.

Malignancy was the most common cause of death, occurring in 12 (55 percent) of the patients (26, 27). Hypertension significantly associated with mortality; 10 of 12 patients with high blood pressure died during the observation period.

NF1 risk factors

Paternal age has been shown to be significantly advanced in sporadic cases of several other autosomal dominant disorders, but whether paternal age is advanced in sporadic cases of NF1 is not clear. A study in Texas (28) recently addressed this question. Paternal age was obtained from the birth certificates of cases (identified as NF1 patients seen in two specialty neurofibromatosis clinics) and birth certificates of controls (two per case, chosen at random from the same year and county of birth). Fathers of NF1 patients were 1.5 years older than were fathers of controls at the birth of the child, but this difference was not statistically significant ($p = 0.07$) (28). It appears that the paternal age effect in sporadic cases of NF1 is either small or nonexistent.

ASSOCIATIONS

NF1 is the condition most commonly associated with *NF1* gene mutations. For NF1, the penetrance is believed to be virtually 100 percent by adulthood (29); that is, individuals with an *NF1* gene mutation have clinical manifestations of NF1, usually by age 6 years. Most studies have not found an obvious relation between particular *NF1* mutations and resulting clinical manifestations in a patient. However, attempts at genotype-phenotype correlation in NF1 are confounded by the effect of age, which increases the frequency of disease manifestations and the likelihood of serious complications in all patients. In addition, there is no consensus regarding how to define NF1 severity.

Some studies of patients with large *NF1* gene deletions indicate that they may have earlier onset of cutaneous neurofibromas and more often have dysmorphic facial features and mental retardation than do most NF1 patients (13, 30, 31). However, not all NF1 patients with this phenotype have a large gene deletion (32), and some with large gene deletions have an unremarkable NF1 phenotype (33), raising questions about this genotype-phenotype relation. The presence of a more severe phenotype may be a function of the amount of flanking DNA involved in the deletion rather than of the *NF1* gene deletion itself.

Certain variants of NF1 have been associated either with specific *NF1* mutations or with linkage to the *NF1* gene, at least in some cases. These include Watson syndrome (characterized by pulmonic stenosis, café-au-lait spots, short stature, and cognitive impairment) (34, 35); familial multiple café-au-lait spots (without other NF1 features) (36–38); familial spinal neurofibromatosis (characterized by spinal tumors and, sometimes, café-au-lait spots, but not by other features of NF1) (39, 40); and encephalocraniocutaneous lipomatosis (characterized by unilateral lipomatous growths, ipsilateral ophthalmologic and brain malformations, mental retardation, and seizures) (41). It appears that these variants may be allelic to NF1, at least in some families.

Patients with segmental neurofibromatosis have features of NF1 confined to a particular area of the body (e.g., one side of the body) (42). While it has been postulated that segmental neurofibromatosis results from a somatic mutation in the *NF1* gene, this postulate has not yet been molecularly demonstrated. Somatic mosaicism for the *NF1* gene has been reported in at least four cases (33, 43–45), but all of these cases showed typical NF1, suggesting that the somatic mutation occurred early in embryonic development.

Noonan syndrome is an autosomal dominant condition characterized by webbing of the neck, unusual facies, short stature, and congenital heart disease (often pulmonic stenosis). Features of Noonan syndrome, often without a cardiovascular malformation, have been observed in many patients with NF1. About 13 percent of patients with NF1 specifically examined for Noonan syndrome features had a Noonan syndrome phenotype (46); this frequency of co-occurrence seems

unlikely if NF1 and Noonan syndrome are independent disorders. In some families, NF1 and Noonan syndrome have been shown to segregate as independent autosomal dominant traits, and Noonan syndrome is not linked to the *NF1* locus in families without features of NF1. In other instances, features of both Noonan syndrome and NF1 appear to result from mutations of the *NF1* gene, and these phenotypes segregate together (46). It appears that the concurrence of NF1 and Noonan syndrome may have several different causes (47), but this question awaits further study.

NF1 and the associated clinical presentations discussed above are the only conditions known to be caused by *NF1* gene mutations. No studies of the *NF1* gene in the general population have been performed.

INTERACTIONS

The wide variability of the NF1 phenotype, even in individuals with the same *NF1* gene mutation, suggests that other factors are involved in determining clinical manifestations. These may include other modifying genes, environmental factors, and chance. Thus far, little is known about the relative contribution of these to the NF1 phenotype.

A study of 175 individuals in 48 families, including six monozygotic twin pairs, evaluated variation of the NF1 phenotype with degree of relation (48). The number of café-au-lait spots and of neurofibromas showed a high correlation between monozygotic twins, a lower correlation between first-degree relatives, and the lowest correlation among more distant relatives. The study also looked at the presence or absence of plexiform neurofibromas, optic gliomas, scoliosis, epilepsy, and referral for remedial education. With the exception of plexiform neurofibromas, these traits also showed familial clustering. The authors concluded that much of the phenotypic variation in NF1 is related to trait-specific "modifying genes."

It has been suggested that environmental factors influence NF1 phenotype; however, no convincing evidence has been presented to support the involvement of any particular environmental factor. Riccardi (49) has suggested that mechanical trauma (in the form of injury to the skin) may often precede the development of neurofibromas, but the evidence for involvement of this factor is anecdotal.

The role of stochastic factors (chance) in the occurrence of some NF1 manifestations has also been hypothesized. Chance may be involved in determining which cells are affected by a somatic mutation and at what point in development somatic mutation occurs. Major questions remain about how the NF1 phenotype is determined, but it is likely that the NF1 genotype, modifying genes, environmental factors, and chance all play a role in the clinical manifestations of *NF1* gene mutations.

LABORATORY TESTS

Laboratory testing for *NF1* mutations is difficult. Although a variety of approaches has been used singly or in combination in research laboratories, none has been shown to be appropriate for routine clinical use.

A protein truncation test is available commercially for *NF1* mutation testing, but its sensitivity, specificity, and positive predictive value in a large group of patients have not been reported. In this test, RNA is reverse transcribed, and the complementary DNA product is used to perform in vitro transcription and translation. Truncated neurofibromin proteins are identified by separating the protein products using an sodium dodecyl sulfate-polyacrylamide gel (50). Mutations may then be confirmed by direct DNA sequencing. False-positive results are possible when truncated proteins are not confirmed by sequencing (16). In addition, the protein truncation test cannot detect mutations that do not result in a truncated protein, such as missense mutations and large deletions, or mutations in which the RNA is unstable and, thus, is unavailable for reverse transcription. The ability of the protein truncation test to detect mosaic mutations is unknown (16). However, it appears that the risk for both false positives (when a finding of a truncated protein is not confirmed by DNA sequencing) and false negatives may be significant with this test. Published studies of the sensitivity of the protein truncation test have been small; about 70 percent of the cases meeting NF1 diagnostic criteria (13 of 20 cases in one study (50) and 11 of 15 cases in another (51)) had a positive result on the protein truncation test. Thirty-seven (77 percent) of 48 cases that met NF1 diagnostic criteria referred for commercial testing are reported to have had a positive protein truncation test result (T. Brown, LabCorp, Research Triangle Park, North Carolina, personal communication, 1999). No information is available on the specificity or positive predictive value of the protein truncation test. When the protein truncation test is negative, further molecular studies may be helpful in identifying the mutation, but these studies are currently available only on a research basis.

In familial NF1 cases (when two or more family members are affected), linkage analysis can be performed. The availability of intragenic microsatellite NF1 markers has increased the proportion of families in which linkage studies will be informative and has also increased the diagnostic accuracy (52) to an average of 90 percent.

Given that NF1 is easily diagnosed clinically in most affected individuals over age 6 years, the need for laboratory testing is limited to specific circumstances. One of these is for prenatal diagnosis when one of the

parents has NF1. If the causative mutation has been identified, direct testing for this specific mutation can be performed on chorionic villus or amniotic fluid samples. However, the severity of NF1 cannot be predicted prenatally; only the presence or absence of the mutation can be identified. Because of the wide variability in NF1 clinical expression, many families do not find prenatal diagnosis of NF1 acceptable (16).

In families in which there are multiple affected relatives, linkage analysis can also be used for prenatal diagnosis. Once again, only the presence or absence of the affected allele can be predicted, not the severity of the clinical manifestations.

The other situation in which laboratory testing may be considered is in children at risk for NF1, before clinical diagnostic criteria are met. The child may be at risk because of a family history or because of having some features (typically café-au-lait spots), but not sufficient features to meet the established diagnostic criteria. While the ability to confirm or rule out the diagnosis with a laboratory test would be helpful, these children are at particular risk for possible stigmatization and unnecessary medical intervention if a false-positive test results (16). Therefore, following the child on a regular basis for appearance of NF1 complications and sufficient clinical criteria to assure the diagnosis is likely to be a better option at this time.

POPULATION TESTING

No general, population-based studies using molecular testing to identify *NF1* mutations have been performed. This type of study seems unnecessary since individuals over age 6 years with *NF1* mutations can usually be identified by physical and ophthalmologic examination.

Clinical methods of NF1 ascertainment have been performed to estimate the prevalence of the condition in research studies in different populations (see Prevalence of NF1, above). However, population-based screening of individuals for clinical features of NF1 has not received substantial support. This is, in part, due to the difficulty of the effort: Careful physical examination for NF1 features is time consuming, unlike other population-based screening methods based on a simple laboratory test. In addition, since many NF1 features are age dependent, diagnosis in a child under age 3 years is often challenging. However, most adult individuals with NF1 can be identified as a result of a regular physical examination, even in the absence of a screening program.

An important question is whether an early NF1 diagnosis, achieved through a screening program, would lead to prevention of NF1 complications. Since primary prevention of NF1 complications is not presently possible, this beneficial effect would be confined to the possibility that early recognition of complications may result in improved treatment. Several studies have assessed whether screening of individuals already known to have NF1 for complications is helpful. A recent paper suggests that the vast majority of abnormalities identified through a comprehensive screening program (consisting of ophthalmologic consultation with slit-lamp examination, chest radiograph, abdominal ultrasonography, neuroimaging, and analysis of catecholamine levels) did not result in therapeutic action (53). Studies such as these have led many NF1 experts to suggest that a careful clinical evaluation for NF1 complications on an annual basis (or more often, if necessary) by a physician familiar with NF1 is optimal for affected individuals (16). Regular ophthalmologic examination is also recommended for children with NF1 (18). Unfortunately, no studies are available that address the more general question of whether an earlier NF1 diagnosis, made through a screening program, would lead to improved treatment.

Another valid concern when considering whether a population-based screening program may be beneficial is the effect that early diagnosis may have on family planning (avoidance of future pregnancies or utilization of prenatal diagnosis). In a recent survey, the majority of parents preferred an early diagnosis of NF1 in their child; however, NF1 diagnosis did not usually result in avoidance of future pregnancies, and while prenatal diagnosis was viewed favorably, only a few parents said they would actually terminate an affected pregnancy (54). All of these issues will need to be taken into account in the discussion regarding population-based screening (whether using molecular methods or clinical methods); however, at this time, it appears that the benefits of early diagnosis do not outweigh the potential costs of a population-based screening program.

REFERENCES

1. Cawthon RM, Weiss R, Xu GF, et al. A major segment of the neurofibromatosis type 1 gene: cDNA sequence, genomic structure, and point mutations. Cell 1990;62:193–201.
2. Wallace MR, Marchuk DA, Anderson LB, et al. Type 1 neurofibromatosis gene: identification of a large transcript disrupted in three NF1 patients. Science 1990;249:181–6.
3. Li Y, O'Connell P, Breidenbach HH, et al. Genomic organization of the neurofibromatosis 1 gene (*NF1*). Genomics 1995;25:9–18.
4. Xu GF, Lin B, Tanaka K, et al. The catalytic domain of the neurofibromatosis type 1 gene product stimulates *ras* GTPase and complements ira mutants of *S. cerevisiae*. Cell 1990;63:835–41.
5. Colman SD, Williams CA, Wallace MR. Benign neurofibromas in type 1 neurofibromatosis (NF1) show somatic deletions of the *NF1* gene. Nat Genet 1995;11:90–2.
6. Side L, Taylor B, Cayouette M, et al. Homozygous inactivation of the *NF1* gene in bone marrow cells from children with neurofibromatosis type 1 and malignant myeloid disorders. N Engl

J Med 1997;336:1713–20.
7. Huson SM, Compston DAS, Clark P, et al. A genetic study of von Recklinghausen neurofibromatosis in southeast Wales. I. Prevalence, fitness, mutation rate, and effect of parental transmission on severity. J Med Genet 1989;26:704–11.
8. Lázaro C, Ravella A, Gaona A, et al. Neurofibromatosis type 1 due to germ-line mosaicism in a clinically normal father. N Engl J Med 1994;331:1403–7.
9. Riccardi VM. Neurofibromatosis: phenotype, natural history, and pathogenesis. 2nd ed. Baltimore, MD: The Johns Hopkins University Press, 1992.
10. Jadayel D, Fain P, Upadhyaya M, et al. Paternal origin of new mutations in von Recklinghausen neurofibromatosis. Nature 1990;343:558–9.
11. Stephens K, Kayes L, Riccardi VM, et al. Preferential mutation of the neurofibromatosis type 1 gene in paternally derived chromosomes. Hum Genet 1992;88:279–82.
12. Lázaro C, Gaona A, Ainsworth P, et al. Sex differences in mutational rate and mutational mechanism in the *NF1* gene in neurofibromatosis type 1 patients. Hum Genet 1996;98:696–9.
13. Upadhyaya M, Ruggieri M, Maynard J, et al. Gross deletions of the neurofibromatosis type 1 (*NF1*) gene are predominantly of maternal origin and commonly associated with a learning disability, dysmorphic features and developmental delay. Hum Genet 1998;102:591–7.
14. Dublin S, Riccardi VM, Stephens K. Methods for rapid detection of a recurrent nonsense mutation and documentation of phenotypic features in neurofibromatosis type 1 patients. Hum Mutat 1995;5:81–5.
15. National Institutes of Health Consensus Development Conference. Neurofibromatosis: conference statement. Arch Neurol 1988;45:575–8.
16. Gutmann DH, Aylsworth A, Carey JC, et al. The diagnostic evaluation and multidisciplinary management of neurofibromatosis 1 and neurofibromatosis 2. JAMA 1997;278:51–7.
17. Friedman JM, Birch PH. Type 1 neurofibromatosis: a descriptive analysis of the disorder in 1728 patients. Am J Med Genet 1997;70:138–43.
18. Listernick R, Louis DN, Packer RJ, et al. Optic pathway gliomas in children with neurofibromatosis 1: consensus statement from the NF1 Optic Pathway Glioma Task Force. Ann Neurol 1997;41:143–9.
19. Stevenson DA, Birch PH, Friedman JM, et al. Descriptive analysis of tibial pseudarthrosis in patients with neurofibromatosis 1. Am J Med Genet 1999;84:413–19.
20. North KN, Riccardi V, Samango-Sprouse C, et al. Cognitive function and academic performance in neurofibromatosis 1: consensus statement from the NF1 Cognitive Disorders Task Force. Neurology 1997;48:1121–7.
21. Sorensen SA, Mulvihill JJ, Nielsen A. Long-term follow-up of von Recklinghausen neurofibromatosis. Survival and malignant neoplasms. N Engl J Med 1986;314:1010–15.
22. Poyhonen M, Niemela S, Herva R. Risk of malignancy and death in neurofibromatosis. Arch Pathol Lab Med 1997;121:139–43.
23. Cohen BH, Kaplan AM, Packer RJ. Management of intracranial neoplasms in children with neurofibromatosis type 1 and 2. The Children's Cancer Study Group. Pediatr Neurosurg 1990–91;16:66–72.
24. Stiller CA, Chessells JM, Fitchett M. Neurofibromatosis and childhood leukaemia/lymphoma: a population-based UKCCSG study. Br J Cancer 1994;70:969–72.
25. Garty BZ, Laor A, Danon YL. Neurofibromatosis type 1 in Israel: survey of young adults. J Med Genet 1994;31:853–7.
26. Zöller M, Rembeck B, Åkesson HO, et al. Life expectancy, mortality and prognostic factors in neurofibromatosis type 1: a twelve-year follow-up of an epidemiological study in Göteborg, Sweden. Acta Derm Venereol 1995;75:136–40.
27. Zöller M, Rembeck B, Oden A, et al. Malignant and benign tumors in patients with neurofibromatosis type 1 in a defined Swedish population. Cancer 1997;79:2125–31.
28. Bunin GR, Needle M, Riccardi VM. Paternal age and sporadic neurofibromatosis 1: a case-control study and consideration of the methodologic issues. Genet Epidemiol 1997;14:507–16.
29. Carey JC, Laub JM, Hall BD. Penetrance and variability in neurofibromatosis: a genetic study of 60 families. Birth Defects Orig Art Ser 1979;15:271–81.
30. Cnossen MH, van der Est MN, Breuning MH, et al. Deletions spanning the neurofibromatosis type 1 gene: implications for genotype-phenotype correlations in neurofibromatosis type 1? Hum Mutat 1997;9:458–64.
31. Leppig KA, Kaplan P, Viskochil D, et al. Familial neurofibromatosis 1 microdeletions: cosegregation with distinct facial phenotype and early onset of cutaneous neurofibromata. Am J Med Genet 1997;73:197–204.
32. Tonsgard JH, Yelavarthi KK, Cushner S, et al. Do *NF1* gene deletions result in a characteristic phenotype? Am J Med Genet 1997;73:80–6.
33. Rasmussen SA, Colman SD, Ho VT, et al. Constitutional and mosaic large *NF1* gene deletions in neurofibromatosis type 1. J Med Genet 1998;35:468–71 .
34. Allanson JE, Upadhyaya M, Watson GH, et al. Watson syndrome: is it a subtype of type 1 neurofibromatosis? J Med Genet 1991;28:752–6.
35. Tassabehji M, Strachan T, Sharland M, et al. Tandem duplication within a neurofibromatosis type 1 (*NF1*) gene exon in a family with features of Watson syndrome and Noonan syndrome. Am J Hum Genet 1993;53:90–5.
36. Charrow J, Listernick R, Ward K Autosomal dominant multiple café-au-lait spots and neurofibromatosis-1: evidence of non-linkage. Am J Med Genet 1993;45:606–8.
37. Brunner HG, Hulsebos T, Steijlen PM, et al. Exclusion of the neurofibromatosis 1 locus in a family with inherited café-au-lait spots. Am J Med Genet 1993;46:472–4.
38. Abeliovich D, Gelman-Kohan Z, Silverstein S, et al. Familial café-au-lait spots: a variant of neurofibromatosis type 1. J Med Genet 1995;32:985–6.
39. Pulst SM, Riccardi VM, Fain P, et al. Familial spinal neurofibromatosis: clinical and DNA linkage analysis. Neurology 1991;41:1923–7.
40. Poyhonen M, Leisti E-L, Kytola S, et al. Hereditary spinal neurofibromatosis: a rare form of NF1? J Med Genet 1997;34: 184–7.
41. Legius E, Wu R, Eyssen M, et al. Encephalocraniocutaneous lipomatosis with a mutation in the *NF1* gene. J Med Genet 1995;32:316–19.
42. Hager CM, Cohen PR, Tschen JA. Segmental neurofibromatosis: case reports and review. J Am Acad Dermatol 1997;37: 864–9.
43. Colman SD, Rasmussen SA, Ho VT, et al. Somatic mosaicism in a patient with neurofibromatosis type 1. Am J Hum Genet 1996;58:484–90.
44. Ainsworth PJ, Chakraborty PK, Weksberg R. Example of somatic mosaicism in a series of de novo neurofibromatosis type 1 cases due to a maternally derived deletion. Hum Mutat 1997;9:452–7.
45. Wu BL, Boles RG, Yaari H, et al. Somatic mosaicism for deletion of the entire *NF1* gene identified by FISH. Hum Genet 1997;99:209–13.
46. Colley A, Donnai D, Evans DGR. Neurofibromatosis/Noonan phenotype: a variable feature of type 1 neurofibromatosis. Clin Genet 1996;49:59–64.
47. Carey JC. Neurofibromatosis-Noonan syndrome. Am J Med Genet 1998;75:263–4.
48. Easton DF, Ponder MA, Huson SM, et al. An analysis of variation in expression of neurofibromatosis (NF) type 1 (NF1): evidence for modifying genes. Am J Hum Genet 1993; 53:305–13.
49. Riccardi VM. Genotype, malleotype, phenotype, and randomness: lessons from neurofibromatosis-1 (NF-1). Am J Hum Genet 1993;53:301–4.
50. Heim RA, Kam-Morgan LNW, Binnie CG, et al. Distribution of 13 truncating mutations in the neurofibromatosis 1 gene. Hum Mol Genet 1995;4:975–81.
51. Park VM, Pivnick EK. Neurofibromatosis type 1 (NF1): a protein truncation assay yielding identification of mutations in 73% of patients. J Med Genet 1998;35:813–20.

52. Lázaro C, Gaona A, Ravella A, et al. Prenatal diagnosis of neurofibromatosis type 1: from flanking RFLPs to intragenic microsatellite markers. Prenat Diagn 1995;15:129–34.
53. Wolkenstein P, Freche B, Zeller J, et al. Usefulness of screening investigations in neurofibromatosis type 1: a study of 152 patients. Arch Dermatol 1996;132:1333–6.
54. Cnossen MH, Smit FJ, de Goede-Bolder A, et al. Diagnostic delay in neurofibromatosis type 1. Eur J Pediatr 1997;156:482–7.
55. Crowe FW, Schull WJ, Neel JV. A clinical, pathological, and genetic study of multiple neurofibromatosis. Springfield, IL: Charles C Thomas, 1956.
56. Sergeyev AS. On the mutation rate of neurofibromatosis. Humangenetik 1975;28:129–38.
57. Samuelsson B, Axelsson R. Neurofibromatosis. A clinical and genetic study of 96 cases in Gothenburg, Sweden. Acta Derm Venereol Suppl (Stockh) 1981;95:67–71.
58. Fuller LC, Cox B, Gardner RJM. Prevalence of von Recklinghausen neurofibromatosis in Dunedin, New Zealand. Neurofibromatosis 1989;2:278–83.
59. Clementi M, Barbujani G, Turolla L, et al. Neurofibromatosis-1: a maximum likelihood estimation of mutation rate. Hum Genet 1990;84:116–18.

APPENDIX 1. INTERNET SITES

General resources

March of Dimes:
http://www.noah.cuny.edu/pregnancy/march_of_dimes/birth_defects/neurofib.html
National Organization for Rare Disorders
http://206.105.18.10/nord/rdb_sum/3.htm

Genetic databases

GeneCards
http://bioinfo.weizmann.ac.il/cards-bin/carddisp?NF1&search=NF1&suff=txt
GeneClinics
http://www.geneclinics.org/profiles/nf1/
Genome Database
http://gdbwww.gdb.org/gdb-bin/genera/accno?GDB:120231
Human Gene Mutation Database
http://www.uwcm.ac.uk/uwcm/mg/search/120231.html
NNFF International NF1 Genetic Mutation Analysis Consortium
http://www.nf.org/nf1gene/
Online Mendelian Inheritance in Man (OMIM).
http://www.ncbi.nlm.nih.gov/htbin-post/Omim/dispmim?162200

Educational resources

Massachusetts General Hospital Neurofibromatosis Clinic
http://neurosurgery.mgh.harvard.edu/NFclinic.htm
National Institute of Neurological Disorders and Stroke
http://www.ninds.nih.gov/patients/disorder/neurofib/neurofib.htm

Support groups

National Neurofibromatosis Foundation
http://www.nf.org/
Neurofibromatosis, Inc.
http://nfinc.org/
Neurofibromatosis
http://touch.ch/neurofibromatosis/Mainfr1.html
The Neurofibromatosis Association
http://www.users.zetnet.co.uk/neurofibromatosis/

Other websites

American Academy of Pediatrics Policy Statement: Health Supervision for Children with Neurofibromatosis
http://www.aap.org/policy/00923.html
World Wide Neurofibromatosis Clinicians Forum
http://www.neurofibromatosis.org/md12.htm

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Mortality in Neurofibromatosis 1: An Analysis Using U.S. Death Certificates

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Although neurofibromatosis 1 (NF1) is a relatively common autosomal dominant condition, information about its effect on mortality is limited. We used Multiple-Cause Mortality Files, compiled from U.S. death certificates by the National Center for Health Statistics, for 1983 through 1997. We identified 3,770 cases of presumed NF1 among 32,722,122 deaths in the United States, a frequency of 1/8,700, which is one-third to one-half the estimated prevalence. Mean and median ages at death for persons with NF1 were 54.4 and 59 years, respectively, compared with 70.1 and 74 years in the general population. Results of proportionate mortality ratio (PMR) analyses showed that persons with NF1 were 34 times more likely (PMR = 34.3, 95% confidence interval [CI] 30.8–38.0) to have a malignant connective or other soft-tissue neoplasm listed on their death certificates than were persons without NF1. Overall, persons with NF1 were 1.2 times more likely than expected (PMR = 1.21, 95% CI 1.14–1.28) to have a malignant neoplasm listed on their death certificates, but the PMR was 6.07 (95% CI 4.88–7.45) for persons who died at 10–19 years of age and was 4.93 (95% CI 4.14–5.82) for those who died at 20–29 years of age. Similarly, vascular disease was recorded more often than expected on death certificates of persons with NF1 who died at <30 years of age (PMR = 3.26, 95% CI 1.31–6.71 at age <10 years; PMR = 2.68, 95% CI 1.38–4.68 at age 10–19 years; and PMR = 2.25, 95% CI 1.46–3.32 at 20–29 years) but not in older persons. This study supports previous findings of decreased life expectancy for persons with NF1 and, within the limitations of death certificates, provides population-based data about NF1 morbidity and mortality that are useful to clinicians caring for patients with NF1.

Introduction

Neurofibromatosis 1 (NF1 [MIM 162200]) is a relatively common autosomal dominant disorder, with a frequency of 1/3,000–4,000 persons (Poyhonen et al. 2000; Rasmussen and Friedman 2000). Cardinal features include multiple café-au-lait spots, benign neurofibromas, and Lisch nodules of the iris. Other common features include learning disabilities, mild shortness of stature, and skeletal abnormalities. An increased risk of malignancy has also been observed in patients with NF1 and may be related to the proposed tumor-suppressor role of the *NF1* gene (Shen et al. 1996).

Despite the high prevalence of NF1, information about its effect on mortality is limited. Sørensen and associates (1986) studied a cohort of 212 patients with NF1 who had been identified 42 years earlier in Denmark. Because probands were identified through hospitals (and therefore may have been more severely affected), the Sørensen group analyzed data on both probands and affected relatives. Survival of people with NF1 was significantly lower than that of the general population, and more so in probands than in affected relatives. Malignant neoplasms were significantly increased, primarily in probands (Sørensen et al. 1986; Neerup and Jensen et al. 1998).

A 12-year follow-up of 70 adult patients with NF1 (Zöller et al. 1995) found a decrease in life expectancy of ~15 years. Malignancy was the cause of death for more than half the patients, and hypertension was significantly associated with mortality. A third study used data from Japanese vital statistics for 1968–1992 on 605 deaths in which neurofibromatosis was listed as the underlying cause of death. The mean age at death in this study was 43 years (Imaizumi 1995). However, the authors did not distinguish between persons with NF1 and neurofibromatosis 2 (NF2), and no data were available on causes of death other than neurofibromatosis. In addition, because only case subjects in which neurofibromatosis was listed as the underlying cause of death were included, neurofibromatosis was believed to be underascertained in this population (Imaizumi 1995).

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In the present investigation, we used data from U.S. death certificates to study rates of NF1-associated deaths. Our population-based study includes data on >3,700 deaths of people with NF1 during 1983–1997. We used these data to examine mean and median ages at death and the most common conditions associated with death in persons with NF1 compared with the general U.S. population.

Methods

We used the Multiple-Cause Mortality Files (MCMFs), which are compiled from U.S. death certificates by the National Center for Health Statistics (NCHS) of the Centers for Disease Control and Prevention. MCMFs include demographic and geographic information and codes from the *International Classification of Disease, Ninth Revision* (ICD9) for the underlying cause of death and for as many as 20 conditions listed on the death certificate as "other significant conditions" (Israel et al. 1986). The ICD9 coding system has been used for mortality statistics since 1979; however, because of incomplete collection of death certificates for 1981 and 1982, the NCHS partially replicated data for these years (Israel et al. 1986). We were concerned about the effect of this case duplication on our small subset of case subjects with neurofibromatosis, so we limited our study to 1983–1997.

The codes in the MCMFs are in two formats: entity axis and record axis. The entity-axis format provides a separate code for each disease listed, whether it is an underlying cause of death or a contributory condition. The record-axis format uses linkage rules to combine some listings of conditions on the death certificates. We selected all cases listing the ICD9 code for "neurofibromatosis (von Recklinghausen's disease)" (237.7) in the record axis. This code may also include cases of the much less common but generally more severe condition NF2. We therefore excluded case subjects with the codes for "sensorineural deafness" (389.1), "benign neoplasms of cranial nerves" (225.1), "benign neoplasms of cerebral meninges"(225.2), and "benign neoplasms of spinal meninges" (225.4) as more likely to have NF2. For the remaining case subjects, we determined mean and median ages at death to approximate the survival of persons with NF1 overall and by sex and race. Race is classified as "white," "black," or "other" in the MCMFs; however, given the small number of case subjects of other races, we grouped subjects into two racial categories, "white" and "other." Because the distribution of age at death was skewed, a logarithmic transformation was applied to these data to obtain the geometric mean age at death and the associated 95% confidence intervals (CIs). We used the nonparametric-median-scores method to test the differences between median age at death in various categories. Version 8.0 of the SAS program was used for all analyses.

To investigate the relation of NF1-associated deaths to other medical conditions, we calculated the proportionate mortality ratio (PMR) for deaths of persons with NF1 for several conditions. The PMR was calculated as the observed number of deaths among persons with NF1 who also had a specific condition divided by the expected number of deaths of all persons with the specific condition. The expected number of deaths associated with a particular condition was calculated on the basis of the proportion of death certificates in the U.S. population listing that condition during 1983–1997, adjusted for decedent's age, sex, race, and death-cohort (Hennekens 1987). Because the complete file containing >32 million deaths was too large for convenient computation, we used a randomly selected subset containing 25% of U.S. deaths during 1983–1997 to calculate the expected number of deaths for each condition. We calculated 95% CIs by assuming that the number of deaths associated with both NF1 and another medical condition was distributed as a Poisson variable (Ahlbom 1993). PMR enables determination of whether a specific medical condition is more or less likely in deaths in which NF1 is listed on the death certificate than in deaths in the general population. If the medical condition is more likely in persons with NF1, the PMR will be >1; if the medical condition is less likely, the PMR will be <1. We selected 90 conditions for PMR analysis, because they are frequently listed as causes of death in the general population or because of their known association with NF1 morbidity or mortality (Sørensen et al. 1986; Zöller et al. 1995; McGaughran et al. 1999). For selected conditions, we also evaluated PMRs by age group at death (<10, 10–19, 20–29, 30–39, 40–49, 50–59, 60–69, or ≥70 years). For this study, we defined vascular disease as hypertensive disease (ICD9 401–405), cerebrovascular disease (ICD9 430–438), or a disease of the arteries or arterioles (ICD9 440–449). This range includes renal artery stenosis (ICD9 440.1) but does not include heart disease, which we considered separately. We defined "heart disease (adult)" as ischemic heart disease (ICD9 410–414), diseases of the pulmonary circulation (ICD9 415–417), and other forms of heart disease (ICD9 420–429).

Results

We identified 3,829 case subjects with the code for neurofibromatosis listed on the death certificate. We excluded 59 case subjects as likely to have NF2. Of these, 24 cases were coded with "benign neoplasms of cranial nerves" (225.1), 32 were coded with "benign neoplasms

of cerebral meninges"(225.2), and 3 were assigned both these codes. No case subjects had been coded for neurofibromatosis and "sensorineural deafness" (389.1) or "benign neoplasms of spinal meninges" (225.4). The mean and median ages at death of the excluded case subjects were 40.1 and 32 years, respectively.

Among 32,722,122 deaths in the United States, 3,770 presumed NF1-associated deaths remained, a frequency of NF1-associated death of 1/8,700 deaths. The mean age at death of persons with NF1 was 15.7 years lower than the mean age at death in the general population (table 1). Although the mean age at death of females with NF1 was >3 years higher than that of males with NF1, the difference between the mean ages at death of persons with NF1 and of the U.S. general population was greater for females. The mean age at death of persons of other races who had NF1 was earlier than that of whites, but the difference between the mean ages at death of persons with NF1 and of the general population was greater for whites than for those of other races. The difference between mean ages at death of persons with NF1 and the general population increased during each of the three 5-year periods studied.

The median age at death of persons with NF1 was 59 years, whereas the median age at death of the U.S. population was 74 years (fig. 1). The median age at death was decreased more, compared with the general population, among females with NF1 than among males with NF1. The median age at death of persons of other races with NF1 was decreased more than that of whites with NF1, compared with the general population. The difference between median ages at death of NF1 case subjects and of the general population increased during each of the three 5-year periods studied.

Because we wondered whether the effect of NF1 mortality was restricted to younger patients, we also evaluated the mean and median age at death of persons who died at age ≥40 years. The mean age at death of persons with NF1 who survived ≥40 years was 65.6 years, whereas the mean age at death for this subset of the U.S. population was 74.5. The median age at death of persons with NF1 who died at age ≥40 years was 67 years, compared with 76 years for the U.S. population.

Malignant neoplasms (all), vascular disease, cerebrovascular disease (a component of vascular disease), scoliosis, epilepsy, and mental retardation were reported more frequently than expected on death certificates of persons with NF1 (table 2). Diabetes mellitus and suicide were reported much less often than expected on death certificates of persons with NF1, and heart disease (adult) and diseases of the arteries and arterioles were reported slightly less often than expected.

Among NF1-associated deaths (table 3), malignant neoplasms of connective and other soft tissue were reported 34 times more frequently than expected, and neoplasms of the brain were reported 5.5 times more frequently than expected. Other neoplasms were less likely to be listed on the death certificates of persons with NF1 than on those of the general population, and this decrease reached statistical significance for malignant neoplasms of the lymphatic/hematopoietic system, breast (in women), trachea/bronchus/lung, pancreas, large bowel, prostate, uterine cervix, and skin. The PMR for all malignant neoplasms, excluding those of connective and other soft tissue and brain, was significantly <1 (PMR = 0.69; 95% CI 0.64–0.75).

Malignant neoplasms as a group occurred more frequently than expected among case subjects <39 years of age (table 4). When connective and other soft-tissue and brain neoplasms were excluded from malignant neoplasms, the PMR for other neoplasms was elevated for persons who died at <20 years of age. The PMR for myeloid leukemia was substantially increased for children aged <10 years, but malignant neoplasms of the lymphatic and hematopoietic system as a group were reported significantly less often than expected among persons with NF1 aged 20–49 years. Malignant neoplasms of connective and other soft tissue were listed much more frequently than expected among persons with NF1 at all ages, and the PMR was >50 for persons who died at age 20–39 years. Malignant neoplasms of the brain were reported more often on death certificates of persons with NF1 at all ages <70 years.

Vascular disease was reported more often than expected among persons with NF1 who were aged <29 years, but not in those who were older. The pattern was similar for cerebrovascular disease considered alone, but the PMR for hypertensive disease increased only among case subjects who died at 20–29 years of age, and the PMR for diseases of the arteries and arterioles decreased among case subjects aged >60 years.

Table 1

Geometric Mean Age, at Death, of Persons with NF1 and of the U.S. General Population, 1983–97

Category	No. of Deaths	Mean Age, at Death, of (years)		Difference between Means [95% CI] (years)
		Persons with NF1	U.S. Population	
All	3,770	54.4	70.1	15.7 [15.0–16.3]
Males	1,874	52.7	66.4	13.7 [12.8–14.6]
Females	1,896	56.1	74.0	17.9 [17.1–18.7]
Whites	3,150	55.4	71.5	16.1 [15.4–16.7]
Other races	620	49.6	61.3	11.7 [9.7–13.4]
1983–87	1,183	54.9	69.2	14.3 [13.2–15.4]
1988–92	1,247	53.9	69.8	15.9 [14.8–17.0]
1993–97	1,340	54.5	71.0	16.5 [15.5–17.6]

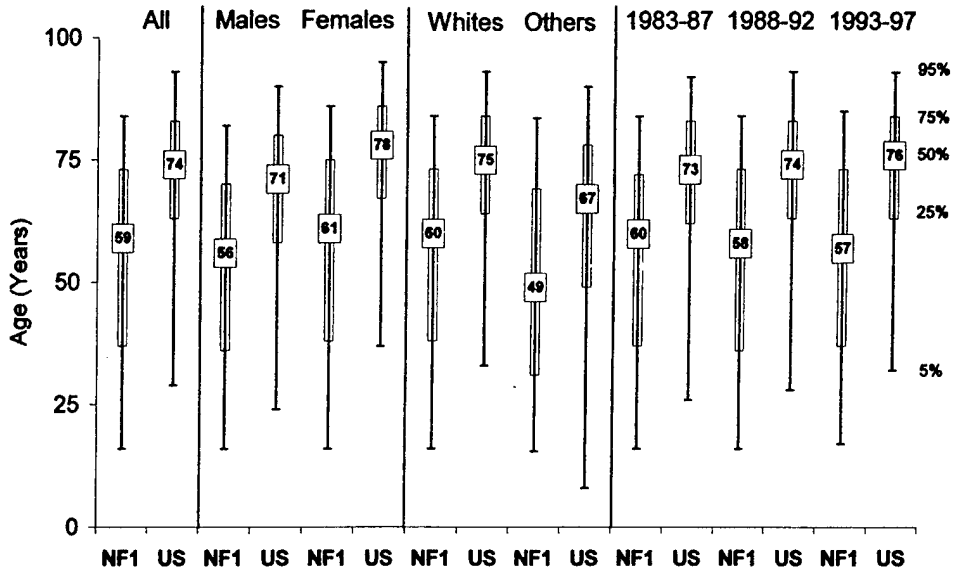

Figure 1 Median (in box) and 5th, 25th, 75th, and 95th percentiles of ages at death of persons with NF1 and of the U.S. general population, for all subjects and by sex, race, and time period.

Discussion

Our finding that survival, as estimated by mean and median age at death, is ~15 years less than expected in persons with NF1 is consistent with the results of previously reported smaller studies. Zöller et al. (1995) monitored 70 adults with NF1 for 12 years and estimated that the mean length of life for persons with NF1 was ~15 years shorter than expected. Inclusion of persons with NF1 presenting in childhood probably would have lowered the life expectancy even further (Zöller et al. 1995). The study by Imaizumi (1995) found that, in a Japanese population, the mean age at neurofibromatosis-associated death was 43 years, much lower than that seen in our study. However, Imaizumi's study included only case subjects for whom "neurofibromatosis" was listed as the underlying cause of death, whereas our study included all case subjects for whom neurofibromatosis was mentioned anywhere on the death certificate and excluded case subjects who were more likely to have NF2 than NF1. Neurofibromatosis was likely to be significantly underascertained in Imaizumi's study, resulting in a larger proportion of severe cases than in ours. Thus, our study population is more likely to be representative of all people with NF1.

Examination of mean and median ages at death suggests that survival of females with NF1 is more severely affected than that of males, in comparison with population expectations. Results of the study by Zöller et al. (1995) also suggested that women with NF1 may be more severely affected than men. In the study by Sørensen et al. (1986), female probands had the lowest survival rate, but the survival of female relatives with neurofibromatosis was only slightly less than that of the general population. Another possible explanation is that NF1 is likely to be diagnosed earlier in females, and thus NF1 may be listed more often on death certificates of females with NF1 who die earlier. In support of this, the frequency of deaths for which NF1 was listed on the death certificate is somewhat greater among females than among males (1/8,343 deaths for females vs. 1/9,019 for males). Further study is needed to determine whether NF1 affects survival more strongly in females than in males.

Evaluating the effect of race on NF1 mortality is more difficult. When subjects are stratified according to race, a comparison of mean age at death of persons with NF1 with mean age at death in the general population suggests that whites may be more severely affected than are other races; however, a comparison of median ages at death suggests that other races are more severely affected. When we compare mean and median ages at death by time period, the effect of NF1 on mortality appears to be increasing. However, these results could be secondary to earlier ascertainment of NF1 cases in more recent years.

The highest PMR in our study was for malignant neoplasms of connective and other soft tissue, a category that includes malignant tumors of the peripheral nerve sheath. These results are consistent with the rarity of such tumors in the general population, their poor prognosis, and their greatly increased frequency among persons with NF1 (Woodruff 1999; King et al. 2000). This type of tumor was identified as a major cause of

Table 2

Likelihood of Selected Medical Conditions Being Listed on NF1-Associated Death Certificates, 1983–97

Condition	ICD9 Code(s)	No. of Cases		PMR (95% CI)
		Observed	Expected	
Malignant neoplasms (all)	140–208	1,170	965	1.21 (1.14–1.28)
Heart disease (adult)	410–414, 415–417, 420–429	1,365	1,571	.87 (.82–.92)
Heart disease (congenital)	745–747	19	19.9	.95 (.57–1.49)
Vascular disease	401–405, 430–438, 440–447	658	597	1.10 (1.02–1.19)
Hypertensive disease	401–405	244	219	1.11 (.98–1.26)
Cerebrovascular disease	430–438	398	327	1.22 (1.10–1.34)
Diseases of arteries and arterioles	440–447	123	157	.78 (.65–.93)
Diabetes mellitus	250	64	239	.27 (.21–.34)
Curvature of spine[a]	737	66	2.72	24.3 (18.8–3.9)
Epilepsy	345	35	10.3	3.40 (2.37–4.73)
Mental retardation	317–319	43	9.00	4.78 (3.46–6.44)
Suicide	E950–E959	7	137	.05 (.02–.11)

[a] Includes scoliosis and kyphosis.

death in previous studies as well. In the study by Zöller et al. (1995), 3 of the 22 patients who died during the period of observation had soft-tissue sarcomas.

We also found a significantly increased PMR for malignant neoplasms of the brain. A significant excess of brain tumors was also found in the study by Sørensen et al. (1986). Of 212 patients with malignant tumors, 21 (10%) had tumors of the CNS; however, some of these persons may have had NF2 (Zöller et al. 1995). A high proportion of brain tumors was also seen in a follow-up study of NF1 patients who had been previously evaluated in a neurofibromatosis clinic (Airewele et al. 2001).

Malignant neoplasms are a major cause of death in people with NF1: ~55% of the cohort studied by Zöller et al. (1995) died of a malignancy, a rate higher than expected on the basis of data from a cancer registry (Zöller et al. 1995). Our study also shows an increased PMR for malignancy among persons with NF1, but the excess was seen only among subjects who died at <40 years of age. This increased PMR appears to result primarily from brain tumors and malignant neoplasms of connective and other soft tissue. When these types of cancer are excluded, the PMR for malignant neoplasms among persons with NF1 is lower than expected (PMR = 0.69; 95% CI 0.64–0.75).

The PMR for myeloid leukemia was significantly elevated among children with NF1 who died at <10 years of age. This observation is consistent with the known relation between juvenile chronic myelogenous leukemia and NF1 and the age at which this malignancy occurs (typically diagnosed at <4 years of age) (Hess et al. 1996). Myeloid leukemia was no more frequent than expected among persons who died with NF1 at other ages.

A number of reports have described life-threatening or fatal vascular abnormalities in young patients with NF1. The most frequently described manifestations are severe hypertension, usually associated with renal artery stenosis, and cerebrovascular disease associated with moyamoya disease (Sobata et al. 1988; Muhonen et al. 1991; Hattori et al. 1998; Kwong and Wong 1999; Fossali et al. 2000). Zöller and colleagues (1995) found that hypertension was significantly associated with NF1 mortality in a series of 70 adult patients monitored for 12 years. PMR for vascular disease was slightly higher than expected among persons with NF1 than among others, and this effect was especially prominent among persons who died at <29 years of age. Most of this increase in the PMR appears to be related to cerebrovascular disease, rather than to hypertensive disease or other diseases of the arteries or arterioles.

On the basis of our evaluation of PMRs by age group, we concluded that the impact of NF1 on mortality from vascular disease and malignancy appears to be focused on persons aged <40 years. However, even among persons with NF1 who survive to age 40, the mean and median ages at death are decreased by ~9 years when compared with the overall U.S. population. This is in contrast to the 15-year decrease observed among all persons with NF1 and suggests that NF1 affects mortality even at older ages, although less so than in earlier years.

This study has several important strengths. The use of MCMFs allows a population-based analysis and a comparison with data from the general population. The study is based on data from recent years, and data are available on deaths of 3,770 people with NF1, many more than all previous studies combined. In addition, our study provides data on deaths at all ages, whereas most previous studies have been limited to deaths among adults.

Our study also has several important limitations. First, the data are based on death certificates, which

Table 3

Likelihood of Selected Malignant Neoplasms Being Listed on NF1-Associated Death Certificates, 1983–97

Type of Malignant Neoplasm	ICD9 Code(s)	No. of Cases		PMR (95% CI)
		Observed	Expected	
Connective and other soft tissue	171.0–171.9	353	10.3	34.3 (30.8–38.0)
Brain	191.0–191.9	181	32.8	5.52 (4.74–6.38)
Lymphatic/hematopoietic system	200–208	69	113	.61 (.48–.77)
Myeloid leukemia	205.0–205.9	20	20.9	.96 (.58–1.48)
Female breast	174.0–174.9	79	120	.66 (.52–.82)
Trachea/bronchus/lung	162.0–162.9	132	236	.56 (.47–.66)
Stomach	151.0–151.9	22	23.5	.94 (.59–1.42)
Liver and intrahepatic bile ducts	155.0–155.9	11	16.1	.68 (.34–1.22)
Pancreas	157.0–157.9	24	39.4	.61 (.39–.91)
Colorectal[a]	153.0–154.9	38	94.6	.40 (.28–.55)
Ovary	183.0	21	28.8	.73 (.45–1.11)
Prostate	185.0–185.9	16	42.6	.38 (.21–.61)
Cervix uteri	180.0–180.9	4	18.7	.21 (.06–.55)
Body (or unspecified part) of uterus	179.0–179.9, 182.0–182.9	11	12.0	.92 (.46–1.64)
Skin (malignant melanoma)	172.0–173.9	12	23.2	.52 (.27–.90)
All neoplasms, excluding connective and soft tissue and brain	140–208, excluding 171 and 191	638	922	.69 (.64–.75)

[a] Includes colon, rectum, rectosigmoid junction, and anus.

previous studies have demonstrated to be both incomplete and, in some instances, inaccurate (Sirken et al. 1987; Lloyd-Jones et al. 1998). The low PMR observed for suicide may be related to this issue; physicians completing death certificates of persons who died of suicide may be less likely to also list NF1. Second, ICD9 does not allow for distinction between NF1 and NF2. We excluded cases coded as neurofibromatosis that had features more characteristic of NF2 than of NF1; but some cases of NF2 probably have been included, and some cases of NF1 may have been excluded from our study. NF1 cases appear to be underascertained in our study population. The proportion of death certificates listing neurofibromatosis is one-third to one-half the estimated population prevalence of NF1 (Poyhonen et al. 2000; Rasmussen and Friedman 2000). This underascertainment could introduce a critical bias if NF1 is more likely to be listed on the death certificates of persons who had severe disease or complications that are well known to be associated with NF1.

In addition, the ICD9 coding system used for mortality statistics is often not specific enough to provide all the information needed for a study of this kind. This limited our analysis of benign tumors; for example, plexiform neurofibromas are designated under the code 215 (other benign neoplasm of connective and other soft tissue), but this code may also include other tumors, such as cutaneous neurofibromas, thereby making analysis of this category impossible.

Another limitation is that our analyses of PMR used multiple comparisons, increasing the likelihood of demonstrating a statistically significant association when one may not exist (Rothman and Greenland 1998). We initially selected 90 codes on which to perform PMR analyses, and we subsequently performed additional analyses on eight age groups. However, the codes were not selected randomly, but focused instead on known associations with NF1 and common causes of death in the general population. It is reassuring that our PMR analyses generally confirm associations that have been previously well recognized in NF1.

Without information about the number of persons with NF1 living in the United States during each year of the study, we cannot be certain that our PMRs actually reflect altered disease-related survival among persons with NF1. A PMR could be lower than expected if competing causes of death eliminated patients who would have developed a particular disease if they had lived long enough (Kupper et al. 1978; Hennekens 1987). However, such effects would have to be differential to affect the PMR; that is, for competing causes of death to produce a lower PMR, persons with NF1 who would have died from one condition later in life would have to die from something else earlier, and persons with NF1 who are unlikely to die from that condition later in life would rarely die from these alternative causes earlier.

Because the PMR is a ratio, an increase in one cause of death results in a decrease in all other causes (Decoufle et al. 1980). The PMR could therefore be reduced if the rate of a particular condition is unchanged but the death rate associated with other medical conditions is greatly increased. The PMR calculation is based on the assumption that the overall rate of death from all causes is the same in the two groups being compared. This is unlikely to be true in the comparison between people with and people without NF1. To the extent that death from all causes is more likely among people with

Table 4

Likelihood of Selected Medical Conditions Being Listed on NF1-Associated Death Certificates, by Age Group, 1983–97

Condition	PMR (95% CI) for Subjects who Died at Age							
	<10 Years	10–19 Years	20–29 Years	30–39 Years	40–49 Years	50–59 Years	60–69 Years	≥70 Years
Malignant neoplasms:								
All	3.94 (2.60–5.73)	6.08 (4.88–7.45)	4.93 (4.14–5.82)	2.13 (1.83–2.47)	1.18 (1.00–1.37)	.89 (.76–1.05)	.77 (.67–.89)	.84 (.74–.96)
Excluding those of connective and soft tissue and brain	3.15 (1.76–5.19)	1.76 (1.07–2.71)	1.27 (.86–1.82)	.55 (.39–.75)	.58 (.45–.73)	.67 (.55–.81)	.59 (.50–.69)	.75 (.65–.87)
Connective and soft tissue	16.1 (5.24–37.6)	38.3 (27.8–51.7)	52.4 (41.8–65.2)	5.2 (4.3–62.0)	31.5 (23.5–41.3)	22.1 (14.7–31.9)	22.9 (15.6–32.3)	18.3 (11.6–27.4)
Brain	3.94 (1.58–8.10)	11.4 (7.53–16.4)	9.32 (6.14–13.5)	7.99 (5.86–1.7)	5.73 (3.96–8.00)	2.99 (1.68–4.94)	3.44 (2.07–5.38)	1.47 (.48–3.42)
Lymphatic and hematopoietic	2.35 (.94–4.84)	.28 (.03–1.01)	.20 (.02–.72)	.07 (.002–.39)	.43 (.16–.93)	.64 (.29–1.22)	.74 (.42–1.20)	.91 (.59–1.33)
Myeloid leukemia	10.4 (3.38–24.3)	1.26 (.15–4.54)	.00 (.00–1.51)	.27 (.007–1.51)	.64 (.08–2.32)	.00 (.00–1.51)	1.20 (.33–3.08)	1.60 (.59–3.48)
Vascular disease:								
All	3.26 (1.31–6.71)	2.68 (1.38–4.68)	2.25 (1.46–3.32)	1.04 (.70–1.50)	1.02 (.75–1.35)	1.12 (.87–1.42)	1.19 (1.00–1.40)	1.01 (.90–1.12)
Hypertensive disease	.00 (.00–28.4)	6.32 (.76–22.6)	3.27 (1.32–6.74)	.32 (.07–.93)	1.07 (.68–1.59)	1.01 (.69–1.43)	1.19 (.91–1.53)	1.13 (.93–1.35)
Cerebrovascular disease	3.63 (1.46–7.47)	2.76 (1.32–5.07)	2.09 (1.19–3.39)	1.20 (.74–1.83)	.85 (.53–1.30)	1.54 (1.12–2.07)	1.46 (1.16–1.81)	1.06 (.92–1.22)
Diseases of arteries and arterioles	.00 (.00–26.3)	.00 (.00–5.51)	2.48 (.80–5.78)	1.15 (.37–2.69)	1.42 (.71–2.55)	.62 (.27–1.23)	.57 (.34–.90)	.78 (.61–.97)

NF1, the PMRs we calculated will underestimate the true cause-specific standardized mortality ratio (Roman et al. 1984). An alternative method that is not subject to this limitation is the use of the standardized mortality odds ratio (SMOR) (Miettinen and Wang 1981) to analyze death-certificate data. We also performed SMOR analyses on these data, and the results were similar to those reported here for PMR (data not shown).

In conclusion, on the basis of our analysis of data from U.S. death certificates, persons with NF1 appear to have a decrease in life expectancy of ~15 years, compared with the general population. However, because NF1 may have been significantly underascertained in our study population, our analysis may overestimate the difference in life expectancy between persons with NF1 and the general population. Certain kinds of malignancy (especially brain tumors and malignant neoplasms of connective and other soft tissues) appear to occur more frequently than expected in people who die with NF1, but other kinds of cancer do not. Such malignancies and vascular disease (especially cerebrovascular disease) appear to contribute disproportionately to mortality in children and young adults with NF1.

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Electronic-Database Information

The accession number and URL for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for NF1 [MIM 162200])

References

Ahlbom A (1993) Biostatistics for epidemiologists. Lewis, Boca Raton, FL

Airewele GE, Sigurdson AJ, Wiley KJ, Frieden BE, Caldarera LW, Riccardi VM, Lewis RA, Chintagumpala MM, Ater JL, Plon SE, Bondy ML (2001) Neoplasms in neurofibromatosis 1 are related to gender but not to family history of cancer. Genet Epidemiol 20:75–86

Decoufle P, Thomas TL, Pickle LW (1980) Comparison of the proportionate mortality ratio and standardized mortality ratio risk measures. Am J Epidemiol 111:263–269

Fossali E, Signorini E, Intermite RC, Casalini E, Lovaria A, Maninetti MM, Rossi LN (2000) Renovascular disease and hypertension in children with neurofibromatosis. Pediatr Nephrol 14:806–810

Hattori S, Kiguchi H, Ishii T, Nakajima T, Yatsuzuka H (1998) Moyamoya disease with concurrent von Recklinghausen's disease and cerebral arteriovenous malformation. Pathol Res Pract 194:363–369

Hennekens CH, Buring JE (1987) Epidemiology in medicine. Little, Brown, Boston, pp 85–86

Hess JL, Zutter MM, Castleberry RP, Emanuel PD (1996) Juvenile chronic myelogenous leukemia. Am J Clin Pathol 105:238–248

Imaizumi Y (1995) Mortality of neurofibromatosis in Japan, 1968–1992. J Dermatol 22:191–195

Israel RA, Rosenberg HM, Curtin LR (1986) Analytical potential for multiple cause-of-death data. Am J Epidemiol 124:161–179

King AA, Debaun MR, Riccardi VM, Gutmann DH (2000) Malignant peripheral nerve sheath tumors in neurofibromatosis 1. Am J Med Genet 93:388–392

Kupper LL, McMichael AJ, Symons MJ, Most BM (1978) On the utility of proportional mortality analysis. J Chronic Dis 31:15–22

Kwong KL, Wong YC (1999) Moyamoya disease in a child with neurofibromatosis type-1. J Paediatr Child Health 35: 108–109

Lloyd-Jones DM, Martin DO, Larson MG, Levy D (1998) Accuracy of death certificates for coding coronary heart disease as the cause of death. Ann Intern Med 129:1020–1026

McGaughran JM, Harris DI, Donnai D, Teare D, MacLeod R, Westerbeek R, Kingston H, Super M, Harris R, Evans DG (1999) A clinical study of type 1 neurofibromatosis in northwest England. J Med Genet 36:197–203

Miettinen OS, Wang JD (1981) An alternative to the proportionate mortality ratio. Am J Epidemiol 114:144–148

Muhonen MG, Godersky JC, VanGilder JC (1991) Cerebral aneurysms associated with neurofibromatosis. Surg Neurol 36:470–475

Neerup Jensen L, Fenger K, Olsen JH, Mulvihill JJ, Sørensen SA (1998) Cancer and mortality in neurofibromatosis 1 (NF1): a 54-year follow-up of a nationwide cohort in Denmark. Am J Hum Genet 63:A114

Poyhonen M, Kytola S, Leisti J (2000) Epidemiology of neurofibromatosis type 1 (NF1) in northern Finland. J Med Genet 37:632–636

Rasmussen SA, Friedman JM (2000) NF1 gene and neurofibromatosis 1. Am J Epidemiol 151:33–40

Roman E, Beral V, Inskip H, McDowall M, Adelstein A (1984) A comparison of standardized and proportional mortality ratios. Stat Med 3:7–14

Rothman KJ, Greenland S (1998) Modern epidemiology. Lippincott-Raven, Philadelphia, pp 225–226

Shen MH, Harper PS, Upadhyaya M (1996) Molecular genetics of neurofibromatosis type 1 (NF1). J Med Genet 33: 2–17

Sirken MG, Rosenberg HM, Chevarley FM, Curtin LR (1987) The quality of cause-of-death statistics. Am J Public Health 77:137–139

Sobata E, Ohkuma H, Suzuki S (1988) Cerebrovascular disorders associated with von Recklinghausen's neurofibromatosis: a case report. Neurosurgery 22:544–549

Sørensen SA, Mulvihill JJ, Nielsen A (1986) Long-term follow-up of von Recklinghausen neurofibromatosis. Survival and malignant neoplasms. N Engl J Med 314:1010–1015

Woodruff JM (1999) Pathology of tumors of the peripheral nerve sheath in type 1 neurofibromatosis. Am J Med Genet 89:23–30

Zöller M, Rembeck B, Akesson HO, Angervall L (1995) Life expectancy, mortality and prognostic factors in neurofibromatosis type 1: a twelve-year follow-up of an epidemiological study in Goteborg, Sweden. Acta Derm Venereol 75: 136–140

Mortality associated with neurofibromatosis 1 in the United States from 1983 to 1995: an analysis using data from death certificates. *S.A. Rasmussen[1], Q.H. Yang[1], J.M. Friedman[2].* 1) Centers for Disease Control and Prevention, Atlanta, GA; 2) Dept. of Medical Genetics, University of British Columbia, Vancouver, BC, Canada.

Neurofibromatosis 1 (NF1) is one of the most common autosomal dominant disorders, occurring in 1 in 3,000 individuals; however, information regarding associated mortality is limited. For this study, we used Multiple-Cause Mortality Files, compiled by the National Center for Health Statistics from US death certificates, for the years 1983 to 1995. These files included International Classification of Diseases, Ninth Revision (ICD9), codes for the underlying cause of death and up to 20 other conditions listed on the death certificate. We selected all cases listing the code for neurofibromatosis. Since this code could also include neurofibromatosis 2 (NF2), which is much less prevalent but usually more severe than NF1, we excluded cases with codes for sensorineural hearing loss or benign neoplasms of the cranial nerves or meninges as probable NF2 cases. 3,253 presumed NF1 cases were identified among over 28 million deaths, a prevalence of 1 in 8,600, suggesting underascertainment of NF1 in this population. Mean and median ages of death for NF1 cases were 54.5 and 59 years, respectively, compared to 69.8 and 74 years in the general population, confirming previous findings of a 15-year decrease in life expectancy among those with NF1. Results of proportionate mortality ratio (PMR) analyses showed that decedents with NF1 were 38 times more likely (PMR=37.9; 95% CI=33.7-42.4) to have a malignant connective or other soft tissue neoplasm listed on their death certificate, compared to those without NF1. Overall, decedents with NF1 were only 1.2 times more likely (PMR=1.2; 95%CI=1.15-1.31) to have a malignant neoplasm than those without NF1. Since previous NF1 mortality studies have suggested that hypertension may be related to mortality, we evaluated hypertensive diseases; these were not more likely to be listed on death certificates of those with NF1 than of those without NF1 (PMR=1.1; 95%CI=0.97-1.28). This study provides population-based mortality data useful to clinicians caring for patients with NF1.

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Descriptive Analysis of Tibial Pseudarthrosis in Patients With Neurofibromatosis 1

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Five percent of individuals with neurofibromatosis type 1 (NF1) present with congenital long bone pseudarthrosis (PA). In large series, 50–80% of patients with congenital long bone PA also have NF1. Very little information exists on the natural history and pathogenesis of PA in NF1. This report is a descriptive analysis of a large series of patients with NF1 and tibial bowing or PA. Study A is a case-control study using the National Neurofibromatosis Foundation International Database (NNFFID). Eighty-five patients with PA were compared to a control group from the same database. There was a statistically significant male predominance of NF1 cases with PA (54 males to 31 females), compared to controls (85 males to 87 females) ($\chi^2 = 4.0$, $P = 0.046$, using a two-tailed test with Yates' correction). There was no significant difference in the clinical presentation of NF1 manifestations in NF1 patients with PA than in NF1 patients without PA. Of the affected individuals with PA, there were 24 de novo cases and 21 familial cases (9 through maternal and 12 through paternal inheritance). Questions that could not be answered by Study A were addressed by a partially overlapping case-series report, Study B, in which data on 75 cases ascertained through questionnaires completed by NF center directors were collected. From Study B we determined that half of the patients who had a fracture sustained it before age 2, and approximately 16% of the pseudarthrosis patients had an amputation. Our data indicate a male predominance and no parent-of-origin effect. Male gender may be a susceptibility factor for pseudarthrosis in NF1. Am. J. Med. Genet. 84:413–419, 1999. © 1999 Wiley-Liss, Inc.

KEY WORDS: neurofibromatosis type 1; pseudarthrosis; tibial bowing; bone dysplasia

INTRODUCTION

Neurofibromatosis type 1 (NF1) is one of the most common genetic disorders of childhood. Among the many associated manifestations of NF1 is the orthopedic complication of long bone pseudarthrosis, usually tibial pseudarthrosis. Ducroquet [1937] first observed that tibial pseudarthrosis is related to NF1. Approximately 5% of patients with NF1, from NF clinics that reported to an international database, have this osseous dysplasia [Friedman and Birch, 1997] and about 50–80% of all reported cases of pseudarthrosis have NF1 [Gilbert and Brockman, 1995; Morrissy et al., 1981; Sofield, 1971]. The literature indicates that NF1 patients with this condition initially present with an-

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terolateral bowing of the long bone [Crawford and Bagamery, 1986; Rudicel, 1987]. Therefore, this type of bowing can be easily distinguished from the mild lateral bowing commonly present in the pediatric population. While the term congenital is usually applied, most patients with or without NF1 present with bowing later than birth, usually in the first year of life. Very little information exists on the natural history and pathogenesis of NF1-related pseudarthrosis, a biologically intriguing and medically challenging condition.

The biologic basis of long bone bowing and pseudarthrosis in NF1 is not known. The bony defects are primary dysplasias and presumably not secondary responses to neurofibromas. While most of the medical manifestations of NF1 involve cells derived from the neural crest, there is no easy explanation for the mesodermally derived osseous defect in NF1.

Our understanding of the natural history of NF1 patients with long bone dysplasia and pseudarthrosis is limited. Such information, if it were available, would be helpful for the management and counseling of these patients. There is no detailed large series study on the natural history of pseudarthrosis in NF1 patients. Some of the questions regarding the biology and natural history of PA in NF1 can be addressed using the National Neurofibromatosis Foundation International Database (NNFFID) [Friedman et al., 1993] and a network of clinic directors. Three specific questions are posed: 1) Are the demographics, clinical manifestations, and developmental history of patients with NF1 different in patients with and without pseudarthrosis? 2) Is there an affected parent-of-origin effect? 3) What is the natural history of tibial bowing and PA in NF1 (i.e., age of onset, age at fracture, number of operations, and rate of amputation)?

METHODS

Information on patients with NF1 is available through the NNFFID. Contributors to the database contribute standard information regarding their NF patients. These data were reviewed and evaluated. In addition, we investigated several natural history questions that are not addressed by the database. A questionnaire was designed to collect information on the natural history of NF1 patients with long bone dysplasia. Thus, this investigation includes two different methods for ascertainment of data. The two components of the study are designated Study A (using the NNFFID) and Study B (using a questionnaire sent to NF clinic directors).

Patient Selection

Study A, the database component, is a case-control study using the NNFFID [Friedman et al., 1993]. This database is a system for collecting comprehensive information on the clinical manifestations of NF. The database currently contains detailed clinical information on individuals contributed by 25 clinics throughout the world. Information is collated in a central database. Confidentiality is maintained by identifying patients by a database number. Local clinics can identify individual patients by linking this database number to the patient's name. Cases with osseous dysplasia of the tibia and/or fibula were selected from the database.

Of the 1,479 unrelated individuals with NF1 included in the database at the time of this analysis, 85 individuals or 5.7% had long bone bowing or pseudarthrosis. Of these, 52 or 3.5% of reported NF1 patients are described as having pseudarthrosis, with the remainder having long bone bowing.

Study B, the questionnaire component, is a case series intended to obtain information on the natural history of pseudarthrosis that was not available in the database. Patient selection consisted of personally contacting NF centers around the world to obtain more specific data on their patients with tibial bowing and pseudarthrosis. Invitations to contribute information on patients were sent to 21 NF centers, of which 10 responded with completed questionnaires.

For confidentiality, centers were asked to use identification numbers instead of names. Thirty patients identified in this survey had identification numbers identical to those of Study A. Using this approach demographic, genetic, and clinical data on 75 patients from various international NF clinics were collected. The data were evaluated for aspects of disease presentation and the presence or absence of certain variables relating to NF1 and pseudarthrosis.

Diagnosis Criteria

Cases with long bone dysplasia who did not meet the NIH criteria for NF1 [Stumpf et al., 1988], were excluded. NIH criteria for the diagnosis of NF1 include at least two of the following findings: six or more café-au-lait spots greater than 5 mm in diameter in prepubertal subjects and greater than 15 mm in postpubertal subjects, two or more neurofibromas or one plexiform neurofibroma, intertriginous freckling, distinctive bone lesions (sphenoid wing dysplasia or pseudarthrosis), two or more Lisch nodules, an optic glioma, or a first-degree relative diagnosed with NF1.

One problem encountered was the difficulty of defining pseudarthrosis and the wide spectrum of associated osseous abnormalities. The classic presentation is tibial bowing (Fig. 1) leading to fracture that results in non-union. However, the spectrum of severity includes simple anterolateral bowing with cortical thickening, hairline fractures, fracture with and without healing after varying times, fibular involvement, amputations, bone grafts, and surgeries before fracture. Various classifications of pseudarthrosis have been published, including Boyd's classification and the more recent classification system by Crawford. None has been widely adopted [Andersen, 1973, 1976a, 1976b; Bassett et al., 1980; Boyd and Sage, 1958; Crawford, 1986; Masserman et al., 1974; McFarland, 1951; Morrissy, 1981; Rathgeb et al., 1974; Sofield, 1971]. The clinician at each referring center determined if cases had any of the above mentioned forms of osseous dysplasia of the tibia and/or fibula. Such cases were included in both Studies A and B.

For this study, two groups were delineated: Group 1 and Group 2. Cases with only simple anterolateral bowing of the tibia or fibula were placed in Group 1.

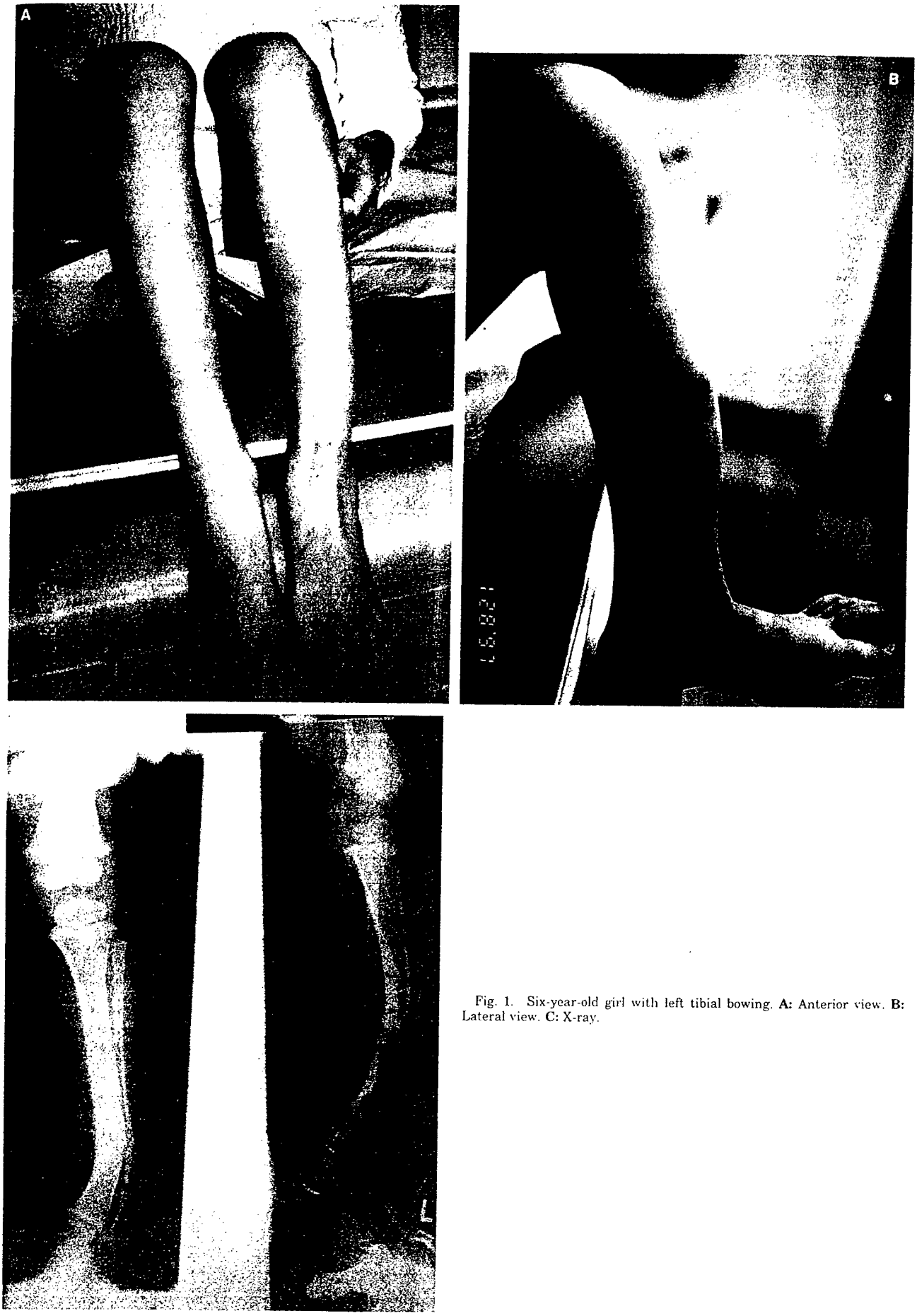

Fig. 1. Six-year-old girl with left tibial bowing. **A:** Anterior view. **B:** Lateral view. **C:** X-ray.

Cases with complications of fracture, pseudarthrosis, surgery, and/or amputation secondary to the bowing were placed in Group 2.

Selection of Control Individuals

Control subjects were only used in Study A. Initially each of the affected individuals from the database was age- and clinic-matched to two control subjects (NF1 patients without bowing/pseudarthrosis). Control subjects were age-matched to pseudarthrosis-affected individuals within 1 year in all cases under 40 years of age. Cases over 40 years of age were matched to within 4 years of control subjects (the oldest individual was 54 years old). Evaluation of the affected patients and their selected matched control subjects identified a few individuals who did not fulfill the NF1 diagnostic criteria. They were eliminated from the study leaving 172 NF1 controls and 85 NF1 individuals affected with pseudarthrosis.

Statistical Analysis

In Study A, NF1 patients with pseudarthrosis were compared to matched NF1 control subjects for gender, mode of inheritance, and associated manifestations. Analysis included Fisher exact tests, Mann-Whitney U tests, and chi-squared calculations using SYSTAT version 5.0.

RESULTS

Study A

Study A analyzed the frequency of 40 different manifestations of NF1 in the control and pseudarthrosis-affected groups. Some include café-au-lait macules, Lisch nodules, discrete neurofibromas, plexiform neurofibromas, optic gliomas, seizures, hydrocephalus, developmental abnormalities, heart disease, endocrine abnormalities, Noonan phenotype, other minor anomalies, and asymmetry unrelated to pseudarthrosis or bowing. Table I summarizes results from a representative sample of some of the more common findings in NF1. When the patients with long bone dysplasia were compared to the NF1 control subjects there was no statistically significant difference in the frequency of any of the 40 features. Likewise, there was no significant difference between trait frequency in Group 1 and Group 2.

Information on whether or not the patient had an affected parent was available in the database on only 53% of cases and 34% of control subjects. Information on maternal versus paternal inheritance was available on all of these familial cases. In Study A, nine people inherited NF1 from their mothers and 12 from their fathers. In the control group, the numbers were 21 and 16 respectively. In this small group there is no statistically significant parent-of-origin effect ($P = 0.41$; Table II).

Most patients were Caucasian in both the control group (82.2%) and the pseudarthrosis-affected group (81.2%); 0.6% of controls and 5.9% of pseudarthrosis-affected cases were of African descent and 6.9% of controls and 9.4% of pseudarthrosis-affected cases were of Asian descent.

There was an excess of affected males (54 males to 31 females). This differed significantly from the control group, in which there were 85 males and 87 females ($\chi^2 = 4.0$, $P = 0.046$, using a two-tailed test with Yates' correction; Fig. 2). Almost the entire difference comes from the male predominance in Group 2 (36 males to 16 females). Group 1 had 18 affected males and 15 affected females.

Study B

The natural history of NF1 patients with long bone dysplasia was addressed through data from a questionnaire. Often the health care provider either did not completely fill out the questionnaire or indicated that the information was not available. For this reason, denominators are not consistent in all categories. Study B included 75 patients. The average age of cases was 11.9 years (N = 72; range = 0.5–54 years, median = 8.6 years). In 53 of the 75 cases the age of bone deformity recognition by a health care provider was established. The mean age of presentation of the osseous problem was 15.3 months (N = 18; range = 0–108 months, median = 8.5 months) in Group 1 and 25.7 months (N = 35; range = 0–228 months, median = 8 months) in Group 2. The combined age of presentation of all cases was 22.2 months (N = 53; range = 0–228 months, median = 8 months). In 36 of 53 patients, in whom the age of recognition of the bone deformity was recognized, the abnormality was identified before one year of age (Table III).

Females averaged fewer operations than males (Table IV). One patient underwent 13 operations while others achieved union with simple casting. In Group 2,

TABLE I. Study A: Common Clinical Manifestations of NF1 With and Without Pseudarthrosis (From NNFFID in Vancouver, BC)

Clinical manifestation	Prevalence in Group 1 (N)	Prevalence in Group 2 (N)	Prevalence in control group (N)	P-Value[a]
≥6 Café-au-lait macules	0.79 (33)	0.88 (52)	0.88 (172)	$P = 0.35$
Intertriginous freckling	0.48 (33)	0.37 (52)	0.29 (172)	$P = 0.08$
Scoliosis	0.18 (33)	0.33 (52)	0.23 (172)	$P = 0.23$
Dysplastic vertebrae	0.09 (33)	0.02 (52)	0.08 (172)	$P = 0.27$
Dysplastic sphenoid wing	0.00 (11)	0.00 (11)	0.07 (45)	$P = 0.55$
Plexiform neurofibroma	0.21 (33)	0.19 (52)	0.24 (172)	$P = 0.71$
Lisch nodules	0.21 (33)	0.35 (52)	0.36 (172)	$P = 0.25$
Glioma	0.03 (33)	0.06 (52)	0.12 (172)	$P = 0.18$
Seizures	0.00 (33)	0.06 (52)	0.05 (172)	$P = 0.40$

[a]2 × 3 Chi-squared analysis looking at differences in distribution across three categories: bowing, complicated, and controls.

TABLE II. Parent of Origin (Study A: Database Component)*

	Familial		
	Maternal	Paternal	De novo
NF1 control (N = 59)	21	6	22
Total affected (N = 45)	9	12	24
Group 1 (N = 18)	2	7	9
Group 2 (N = 27)	7	5	15

*No information available on 153 individuals (cases plus control subjects). Familial vs. de novo: (Group 1/Group 2): Fisher exact test (two-tailed) $P = 0.77$; (Control/total affected): Fisher exact test (two-tailed) $P = 0.12$. Maternal vs. Paternal: Fisher exact test $P = 0.41$.

TABLE III. Age Bony Deformity Recognized (Study B: Questionnaire Component)

	No. of cases	
Age (years)	Group 1	Group 2
At birth	3	7
0–1	10	15
1–2	3	4
2–3	1	3
>3	1	6
Total (N = 532)	18	35

16% (8/50) had an amputation. The average number of operations in Group 2 was 2.9 (range 0–13).

The average age of fracture in pseudarthrosis-affected cases was 4.61 years (N = 32) with a range of 0–28 years with females fracturing an average of 1.06 years later than males (Table IV). In Group 2, 53% fractured before the age of 2 years (Table V).

It was noted that 43% of cases had fibular dysplasia. Two patients had fibular dysplasia without tibial dysplasia. The spectrum of fibular dysplasia ranged from simple bowing to frank pseudarthrosis. Three patients had forearm deformities. They consisted of left ulnar pseudarthrosis with left radial bowing, isolated right radial pseudarthrosis, and right radial and ulnar bowing without pseudarthrosis. The remainder of cases had unilateral tibial and/or fibular deformities. Data analysis was restricted to long bone dysplasia of the tibia and fibula excluding ulnar and radius pseudarthroses.

Laterality of the affected bone was evenly distributed with 35 patients presenting on the left side and 34 patients presenting on the right side. All subjects presented unilaterally. Only three patients out of 71 had a neurofibroma near the site of the deformity.

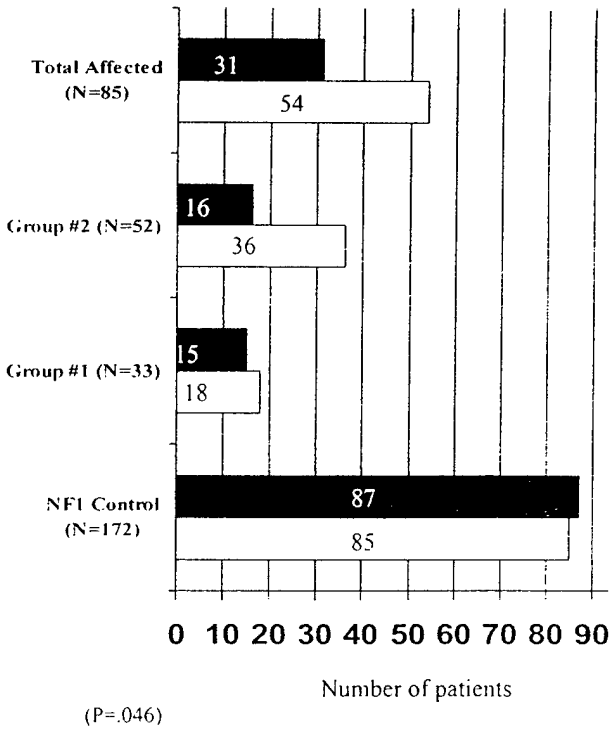

Fig. 2. Study A (database component) gender distribution (females, solid bars; males, open bars).

DISCUSSION

It is known that patients with NF1 exhibit a wide variety of manifestations. This study examined a number of variables with respect to pseudarthrosis (Table I). In Study A, there was no significant difference in the frequency of other clinical manifestations between pseudarthrosis-affected individuals and the control NF1 group. Morrissy et al. [1981] reported an increased observation of gliomas of the central nervous system among individuals with NF1 and pseudarthrosis. This study did not find an association of optic gliomas or neoplasms of any kind among patients.

We identified certain aspects of the natural history of pseudarthrosis in NF1, which may help practitioners more appropriately advise their patients on the potential complications. There are controversies concerning treatment of bowing/pseudarthrosis, and various investigators report different approaches to therapy. Some report one surgical procedure to be better than other procedures [Boyd and Sage, 1958; Charnley, 1956; Farmer, 1952; McFarland, 1951; Moore, 1949; Morrissy et al., 1981; Paterson et al., 1980; Wilson, 1941]. Some claim that amputation should not be done [Sofield, 1971; Van Nes, 1966] while others recommend amputation [Aitken, 1959; Boyd and Fox, 1948; Rathgeb et al., 1974; Rudicel, 1987]. Some patients seek amputation for therapy [Andersen, 1976b; Morrissy, 1981; Van Nes, 1966] due to the many complications that leave them incapacitated and disabled. Orthopedic surgeons remain frustrated on how best to handle this difficult condition. There has been much discussion on the various surgical procedures available to the orthopedist for management of long bone pseudarthrosis. With respect to bowing, the prevention of a fracture seems paramount. Some patients elected to have the bowed bone operated on before a fracture occurs, making it difficult to readily assess when or if a fracture would occur. Several surgical procedures including bone grafting, osteotomy, and vascularized autogenous grafts have been performed. Chronic bracing with a knee-ankle-foot orthosis (KAFO) is advocated by some orthopedists as a modality to prevent bowing from progressing to frank pseudarthrosis [Crawford, 1986]. At the present time there is no standardized protocol or

TABLE IV. Gender Observation of Fracture Age and Operations (Study B: Questionnaire Component)*

	Male	Female	Total	
Average age at fracture (years)	4.4	5.4	4.6	(Range = 0–28 yrs)
	(N = 24)	(N = 8)	(N = 32)	
	(Median = 2.0)	(Median = 2.5)	(Median = 2.0)	
Average no. of operations	3.3	1.8	2.9	(Range = 0–13)
	(N = 28)	(N = 10)	(N = 38)	
	(Median = 2.5)	(Median = 2.0)	(Median = 2.0)	

*Mann-Whitney U test: age at fracture: $P = 0.67$; number of operations: $P = 0.15$.

controlled clinical trial that has rigorously shown to be of value.

This study showed that half of the cases that fractured did so before the age of 2 years. However, the age was highly variable, ranging from prenatal to 28 years. Fifty-nine percent of Group 1 (patients with simple anterolateral bowing) were over the age of 4.61 years (our calculated average age of first fracture) with the oldest being 15.3 years old. This leaves nine patients with bowing who had not reached the average age at which fractures occurred.

It is commonly thought that NF1 patients with tibial bowing will inevitably sustain a fracture. Since this is a retrospective cross-sectional investigation, we were unable to determine from our data if these patients will fracture, but our data suggests that there are patients with significant bowing who may never fracture.

Fibular dysplasia often occurred with tibial dysplasia. In Study B, 43% of cases had fibular dysplasia. Two patients had fibular dysplasia without tibial dysplasia. Long bone dysplasia in NF1 is commonly referred to as tibial pseudarthrosis, but the fibula is often concurrently involved. Pseudarthroses of other long bones besides the tibia and fibula have been reported in NF1 patients. Other affected bones include the ulna, radius, humerus, femur, and clavicle [Rudicel, 1987]. Only a few isolated cases of these pseudarthroses have been reported.

We observed three patients with forearm deformities. They consisted of a case with left ulnar pseudarthrosis and left radial bowing, a case with right radial pseudarthrosis, and a case with right radial and ulnar bowing. None of these three cases had involvement of the tibia.

All cases presented with unilateral deformities. Laterality of the affected bone was evenly distributed in Study B. This observation suggests that other factors play a role in the development of this skeletal dysplasia, and that the NF1 mutant allele is not sufficient to cause the osseous dysplasia. These other factors could likely be somatic mutations of modifier genes.

The pathophysiology of pseudarthrosis is unknown. It has been postulated that a neurofibroma at the site of the deformity actually causes the deformity. Green and Rudo [1943] reported a histological specimen with a neurofibroma growing in the pseudarthrosis segment. Another study by Aegeter [1950] claimed that the tissue surrounding the site of the pseudarthrosis was the cause of the bony deformity. Brooks and Lehman [1924] proposed that the neurofibromas may arise from the nerves of the periosteum, erode into the bone, and then become covered by a shell of bone. However, Crawford and Bagamery [1986] stated that few surgical specimens have neurofibromatous tissue at the pseudarthrosis site and Moore [1941] found no report of actual invasion of the shaft by the neurofibroma. Our study confirms the latter observations. Only three patients of 71 had a neurofibroma near the site of the deformity. These may be incidental or part of the intrinsic dysplasia. Our data do not support the notion of a neurofibroma causing this osseous dysplasia.

There has been some speculation about a parent-of-origin effect. Miller and Hall [1978] noted an increased occurrence of serious complications such as pseudarthrosis when NF1 had been inherited from the mother as opposed to the father. These serious complications included pseudarthrosis. From their study it was postulated that there may be a parent-of-origin effect in the development of pseudarthrosis in patients with NF1. Study A did not suggest a maternal influence, but the numbers are small. There may be a maternal influence in other severe manifestations of NF1; however, these data do not support a parent-of-origin effect in the occurrence of pseudarthrosis (Table II).

Regarding racial distribution, it has been noted that few black Americans have NF1-associated optic nerve gliomas [Saal et al., 1995]. Therefore, there may be a correlation between ethnic origin and various NF1 manifestations. Due to the small number of non-Caucasians (17.8% of controls and 18.8% of affected individuals were non-Caucasian or "unknown"), no statistically significant conclusions could be made regarding racial distribution in this study. It is important to evaluate a larger number of patients of African and Asian descent to determine if the prevalence of pseudarthrosis in NF1 individuals from different ethnic backgrounds varies.

The most striking and singular observation of this study was the gender difference. In Study A, the control group showed an equal distribution of males and

TABLE V. Age at Fracture in Group 2 (Study B: Questionnaire Component)*

Age (years)	No. of cases
At birth	2
0–1	9
1–2	6
2–3	4
3–6	3
6–13	5
>13	3

*(N = 32); range, 0–28 years; mean, 4.6 years.

females, which is consistent with the literature on NF1 patients. In contrast, we found a significant excess of males with long bone dysplasia, especially in Group 2. The natural history study showed that male patients in Group 2 averaged more surgeries with an earlier age of fracture than females (Table IV).

This series is the largest investigation of patients with pseudarthrosis and NF1 reported to date. Previous studies do not provide information on gender, or include fewer patients. Although Gilbert and Brockman [1995] reported that males had a longer healing time than females, and Moore [1957] reported a slight male predominance in pseudarthrosis, neither study differentiated pseudarthrosis patients with NF1 and those without. We found no evidence in the literature to refute our findings.

The observation of a male predominance in the complicated group suggests that male gender could be a susceptibility factor for pseudarthrosis in patients with tibial bowing and NF1. This observation is also noted in NF1-associated leukemias. An increased proportion of males has also been observed among NF1 patients with myelogenous dysplasia [Shannon et al., 1992]. Conceivably, the mesodermal derivation of the tissue of origin of bone marrow cells and skeleton plays a role in the male susceptibility.

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REFERENCES

Aegeter E. 1950. The possible relationship of neurofibromatosis, congenital pseudarthrosis and fibrous dysplasia. J Bone Joint Surg Am 32:618–626.

Aitken GT. 1959. Amputation as a treatment for certain lower extremity congenital abnormalities. J Bone Joint Surg Am 41:1267.

Andersen KS. 1973. Radiological classification of congenital pseudarthrosis of the tibia. Acta Orthop Scand 44:719–727.

Andersen KS. 1976a. Congenital pseudarthrosis of the tibia and neurofibromatosis. Acta Orthop Scand 47:108–111.

Andersen KS. 1976b. Congenital pseudarthrosis of the leg. J Bone Joint Surg Am 58:657–662.

Bassett CAL, Caulo N, Korte GJ. 1980. Congenital pseudarthrosis of the tibia: treatment with pulsing electromagnetic fields. Clin Orthop 154: 136–149.

Boyd HB, Fox KW. 1948. Congenital pseudarthrosis. J Bone Joint Surg 30:274–283.

Boyd HB, Sage FP. 1958. Congenital pseudarthrosis of the tibia. J Bone Joint Surg Am 40:1245–1270.

Brooks B, Lehman EP. 1924. The bone changes in Recklinghausen's neurofibromatosis. Surg Gynecol Obstet 38:587–595.

Charnley J. 1956. Congenital pseudarthrosis of the tibia treated by the intramedullary nail. J Bone Joint Surg Am 38:283–290.

Crawford AH. 1986. Neurofibromatosis in children. Acta Orthop Scand 57:7–60.

Crawford AH, Bagamery N. 1986. Osseous manifestations of neurofibromatosis in childhood. J Pediatric Orthop 6:72–88.

Ducroquet RL. 1937. A propos des pseudoarthroses et inflexions congénitales du tibia. Mem Acad Chir (Paris) 63:863–868.

Farmer AW. 1952. The use of a composite pedicle graft for pseudarthrosis of the tibia. J Bone Joint Surg Am 34:591–600.

Friedman JM, Birch P. 1997. Type 1 neurofibromatosis: a descriptive analysis of the disease in 1728 patients. Am J Med Genet 70:138–143.

Friedman JM, Birch P, Greene C, NNFF International Database Participants. 1993. National neurofibromatosis foundation international database. Am J Med Genet 45:88–91.

Gilbert A, Brockman R. 1995. Congenital pseudarthrosis of the tibia. Clin Orthop 314:37–44.

Green WT, Rudo N. 1943. Pseudarthrosis and neurofibromatosis. Arch Surg 46:639–651.

Masserman RL, Peterson HA, Bianco AJ. 1974. Congenital pseudarthrosis of the tibia: a review of the literature and 52 cases from the Mayo Clinic. Clin Orthop 99:140–145.

McFarland B. 1951. Pseudarthrosis of the tibia in childhood. J Bone Joint Surg Br 33:36–46.

Miller M, Hall JG. 1978. Possible maternal effect on severity of neurofibromatosis. Lancet 2:1071–1073.

Moore BH. 1941. Some orthopaedic relationships of neurofibromatosis. J Bone Joint Surg 43:109–140.

Moore JR. 1949. Delayed autogenous bone graft in the treatment of congenital pseudarthrosis. J Bone Joint Surg Am 31:23–29.

Moore JR. 1957. Congenital pseudarthrosis of the tibia. Instr Course Lect 14:222–237.

Morrissy RT, Riseborough EJ, Hall JE. 1981. Congenital pseudarthrosis of the tibia. J Bone Joint Surg Br 63:367–375.

Paterson DC, Lewis GN, Cass CA. 1980. Treatment of congenital pseudarthrosis of the tibia with direct current stimulation. Clin Orthop 148: 129–135.

Rathgeb JM, Ramsey PL, Cowell HR. 1974. Congenital kyphoscoliosis of the tibia. Clin Orthop 103:178–190.

Rudicel S. 1987. The orthopaedic manifestations of neurofibromatosis. Conn Med 51:221–222.

Saal HM, Schorry EK, Lovell AM, Ball W, Egelhoff J, Koch B, Samango-Sprouse CA, Rosenbaum KN, Stern HJ, Tifft CJ, Vezina LG. 1995. Racial differences in the prevalence of optic gliomas in neurofibromatosis. Am J Med Genet Suppl 57:A54.

Shannon KM, Watterson J, Johnson P, O'Connell P, Shah N, Steinherz P, Kan YW, Priest JR. 1992. Monosomy 7 myeloproliferative disease in children with neurofibromatosis, type 1: epidemiology and molecular analysis. Blood 79:1311–1318.

Sofield HA. 1971. Congenital pseudarthrosis of the tibia. Clin Orthop 76: 33–42.

Stumpf DA, Alksne JF, Annegers JF, Brown SS, Conneally PM, Housman D, Leppert MF, Miller JP, Moss ML, Pileggi AJ, Rapin I, Strohman RC, Swanson LW, Zimmerman A. 1988. Neurofibromatosis conference statement national institutes of health consensus development conference. Arch Neurol 45:575–578.

Van Nes CP. 1966. Congenital pseudarthrosis of the leg. J Bone Joint Surg Am 48:1467–1483.

Wilson PD. 1941. A simple method of two-stage transplantation of the fibula for use in cases of complicated and congenital pseudarthrosis of the tibia. J Bone Joint Surg 23:639–675.

Growth in North American white children with neurofibromatosis 1 (NF1)

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Abstract

***Objective*—To analyse the distributions of and generated growth charts for stature and occipitofrontal circumference (OFC) in neurofibromatosis 1 (NF1) patients.**

***Design*—Cross sectional database survey.**

***Setting*—The National Neurofibromatosis Foundation International Database (NFDB) includes clinical information on NF1 patients from 14 participating centres in North America.**

***Subjects*—A total of 569 white, North American, NF1 patients, 55% female and 45% male.**

***Main outcome measures*—Stature and OFC measurements of NF1 patients were compared to age and sex matched population norms using z score standardisation and centile curves.**

***Results*—The distributions of stature and OFC are shifted and unimodal among NF1 patients; 13% of patients have short stature (≥2 standard deviations below the population mean) and 24% have macrocephaly (OFC ≥2 standard deviations above the population mean).**

***Conclusions*—Alterations of stature and OFC are not limited to NF1 patients with frank short stature or macrocephaly. Keywords: neurofibromatosis 1; stature; occipitofrontal circumference; macrocephaly**

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Neurofibromatosis 1 (NF1) is an autosomal dominant disorder affecting about 1 in 3000 people.[1-3] Its most frequent features are café au lait macules, iris Lisch nodules, and discrete and plexiform neurofibromas. Short stature (≥2 SD below the population mean) and macrocephaly (≥2 SD above the population mean) are more common in people affected with NF1 than in the general population.[4-7]

NF1 features such as scoliosis and early or delayed puberty occasionally influence stature. However, short stature associated with NF1 usually affects the whole skeleton proportionately, and no specific cause is apparent in most cases.[5 8] Disease features such as hydrocephalus and plexiform neurofibromas occasionally affect measurements of occipitofrontal circumference (OFC_J. However, increased OFC among NF1 patients usually has no obvious cause and appears to result from overgrowth of the brain.[5 8]

It has been suggested that short stature and macrocephaly are "all or none" phenomena that affect only a subset of NF1 patients.[5] According to this hypothesis, NF1 patients would be expected to fall into two distinct groups: (1) those whose stature is in the same normal distribution as unaffected people of the same age, and (2) those whose stature is decreased. NF1 patients would also be expected to fall into two distinct groups with respect to macrocephaly: (1) those whose OFC is in the same normal distribution as unaffected people of the same age, and (2) those whose OFC is increased. We examined the distributions of these measurements to determine whether changes in growth affect all or only a subset of patients with NF1. We also generated centile curves for stature and OFC by age and gender.

Subjects and methods

SUBJECTS

All patients included in this study meet the NIH Diagnostic Criteria for NF1.[9 10] Measurements of patient stature and OFC were obtained from the National Neurofibromatosis Foundation International Database (NFDB).[11] At the time of this analysis, the NFDB included extensive demographic and cross sectional clinical and anthropometric data on 569 North American, white, NF1 patients examined during 1980-1998 at 14 participating centres in North America. Information was collected and recorded on each patient using a standard procedure. Patient stature was measured without shoes using a stadiometer. OFC was measured at the largest diameter over the occiput and forehead using an inextensible tape line measure. The data were subjected to automated range checking and routinely screened for quality and consistency by the database administrator. Only measurements from each patient's first visit to a participating clinic were included in the analysis. Patients who were known to have one or more of the following

features on any clinical visit were excluded from analyses of stature: pseudarthrosis (n=22, 3%), early (under 10 years) (n=13, 2%) or delayed (over 15 years) (n=51, 1%) puberty, optic glioma (n=66, 9%), scoliosis (n=98, 14%), or vertebral dysplasia (n=19, 3%). The final sample for analyses of stature consisted of 183 males and 202 females. Patients with one or more of the following features were excluded from analyses of OFC: plexiform neurofibroma of the head (n=46, 6%), early or delayed puberty, optic glioma, or hydrocephalus (n=23, 3%). The ultimate sample for analyses of OFC consisted of 216 males and 220 females.

REFERENCE POPULATIONS

Standard population norms for stature by age were obtained from the National Center for Health Statistics (NCHS) studies during 1963-1974.[12] The NCHS standards are based on a sample consisting of 83% percent white or Hispanic subjects and 17% percent black subjects living in the United States. Standard population norms for OFC by age were obtained from the Fels Institute study conducted during 1929-1975.[12] The Fels Institute sample is slightly less heterogeneous than the NCHS sample.

DISTRIBUTION ANALYSIS

Stature and OFC measurements were standardised using z scores to control for both age and gender:

$$z = \frac{\text{(measurement of patient)} - \text{(mean of the sex and age matched control group)}}{\text{Standard deviation of the sex and age matched control group)}}$$

Patients with stature and OFC measurements corresponding to a z score with an absolute value greater than 7 were excluded to minimise data entry errors. Four (1%) stature and two (0.5%) OFC measurements corresponded to z scores below −7. One (0.3%) stature and two (0.5%) OFC measurements corresponded to z scores above 7. Single data entry, as used in this study, has an error rate around 2%.[13–15] After these exclusions, we expect that about 1% of the remaining measurements contain errors.

We tested the standardised data by analysis of variance to determine if significant differences exist among the measurements made by the major contributing centres.

Distributions of z scores for stature and OFC were plotted in histograms using SAS.[16] Each histogram is based on the z scores compiled from males and females of all ages. In addition, the deviation from unimodality of each distribution was quantified by computing its dip statistic.[17] Dip approaches zero for unimodal distributions. The significance of a given dip value is determined by comparing it to the distribution of values from a known unimodal distribution.

GROWTH CURVES

Centiles were generated directly from the data for NF1 patients of various ages and compared to the corresponding centiles from reference populations. NF1 patients were divided into sex and age groups matching those of the curves available for population norms. A typical series of age groups had medians of 2, 2.5, 3 . . .18 years. Age group limits were determined by splitting the difference between a given median and the next lowest and highest medians. Patients with ages equidistant from two medians were assigned to the older age group. For example, the 2.5 year old group included patients aged 2.250 to 2.749 years old. The 5th, 25th, 50th, 75th, and 95th centiles for stature and OFC were determined for each sex in each age group of NF1 patients and plotted alongside the centiles from the corresponding population standards. The data were plotted and smoothed using SAS.[16] Smoothing was done by producing a cubic spline that minimises a linear combination of the sum of squares of the residuals of fit and the integral of the square of the second derivative.[16 18] Smoothed curves were inspected to ensure that the final results reasonably represent the data. Splining was used and described in detail by Hamill *et al*[12] to generate standard curves for the NCHS.

Results

A total of 183 males and 202 females were included in analyses of stature and 216 males and 220 females were included in analyses of OFC. Analysis of variance for heterogeneity among the 14 North American contributing centres showed no centre bias for age and sex standardised stature (p=0.72) or OFC (p=0.10).

Figs 1 and 2 show the distributions of standardised measurements of stature and OFC among NF1 patients and population norms. Mean standardised stature among NF1 patients is lower than the mean in the reference population. Thirteen percent of the NF1 patients lie 2 SD or more below the reference population mean, compared to 2% of norms. Mean standardised OFC among NF1 patients is greater than the mean in the reference population. Twenty four percent of NF1 patients lie 2 SD or more above the reference population mean.

The histograms for stature and OFC appear unimodal (figs 1 and 2); their dip statistics, which measure departures from unimodality, are 0.014 and 0.012, respectively. These correspond to the 5th centiles of departures from known unimodal distributions. In other words, the deviations are either within the normal range for known unimodal distributions or lower. The standardised stature distribution has a skewness of 0.32 and a kurtosis of 0.19; most of the cases are clustered to the left of the mean and the distribution peaks more abruptly than a normal distribution. The standardised OFC distribution has a skewness of −0.16 and a kurtosis of 0.87; most of the cases are to the right of the mean and the distribution peaks more abruptly than normal.

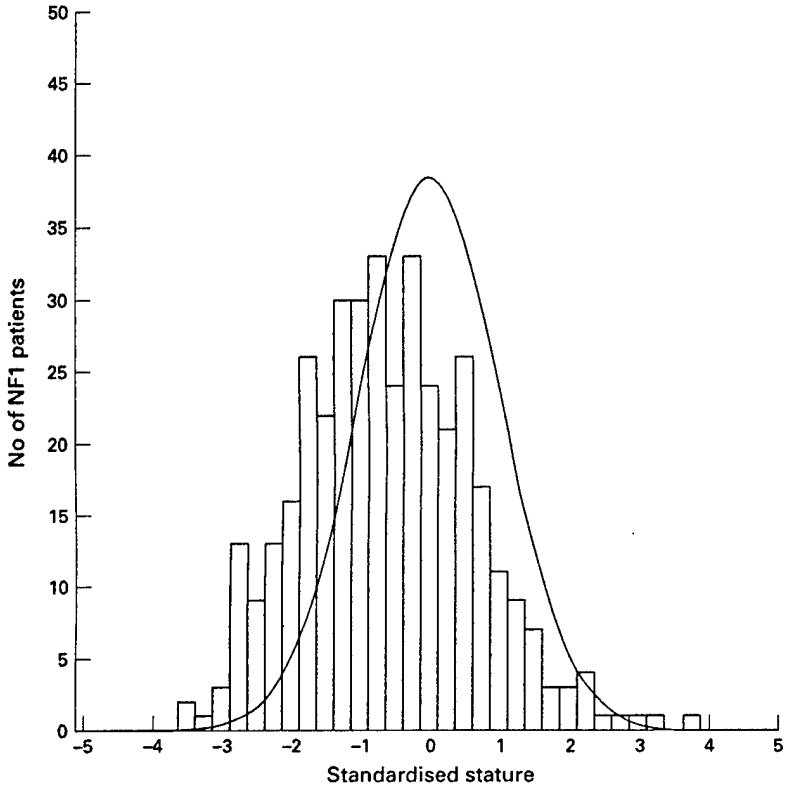

Figure 1 Distribution of sex and age standardised stature. NF1 patient measurements are from the National NF Foundation Database. Unaffected norms are from the National Center for Health Statistics and the Fels Institute.

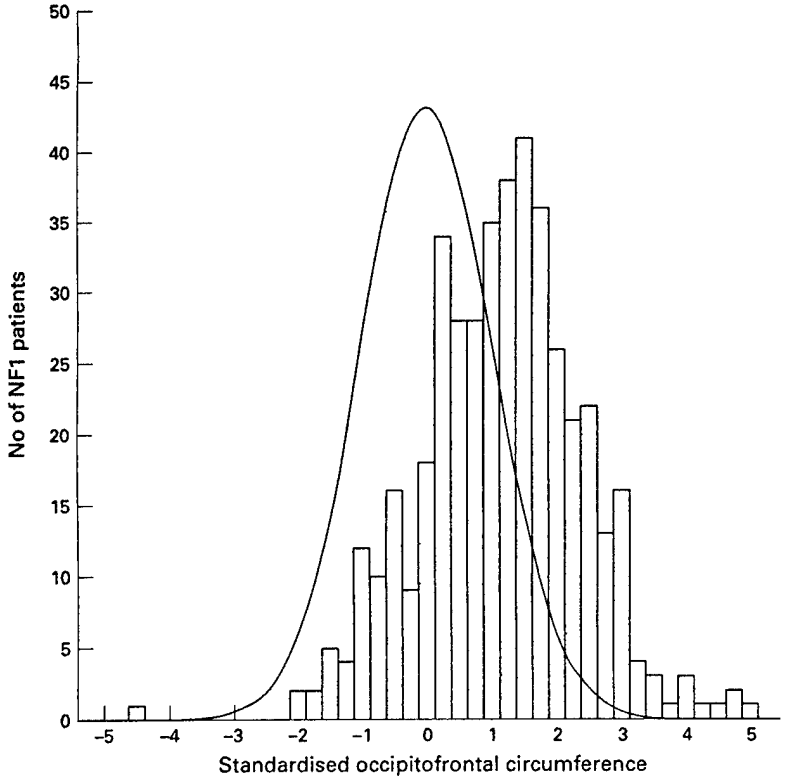

Figure 2 Distribution of sex and age standardised occipitofrontal circumference. NF1 patient measurements are from the National NF Foundation Database. Unaffected norms are from the National Center for Health Statistics and the Fels Institute.

Stature and OFC centiles by age are shown in figs 3 and 4. Median stature is as much as 7 cm lower and OFC 2 cm greater in our NF1 patients than in the standard paediatric growth charts, depending on age and gender.

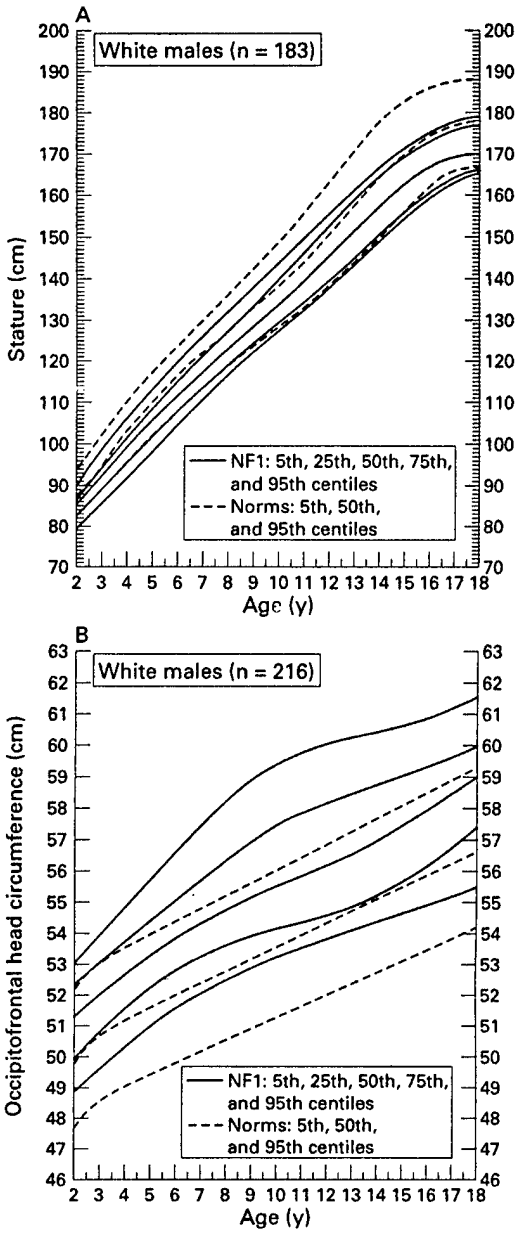

Figure 3 (A) Stature centiles in males 2-18 years. NF1 patient measurements are from the National NF Foundation Database and are denoted by solid lines. Unaffected norms are from the National Center for Health Statistics and the Fels Institute and are denoted by dashed lines. (B) Occipitofrontal circumference centiles in males 2-18 years. NF1 patient measurements are from the National NF Foundation Database and are denoted by solid lines. Unaffected norms are from the National Center for Health Statistics and the Fels Institute and are denoted by dashed lines.

Discussion

The NCHS and Fels standards were used for comparison to stature and OFC of NF1 patients because these studies cover a wide range of ages and are commonly used clinically to diagnose short stature and macrocephaly. These normal population studies were longitudinal and therefore more accurately represent growth than the cross sectional studies we used. More recent NCHS standards for boys and girls are available for stature, but not for occipitofrontal circumference (http://www.cdc.gov/growthcharts). These new stature standards for boys and girls are remarkably similar to the ones we used.[12 19]

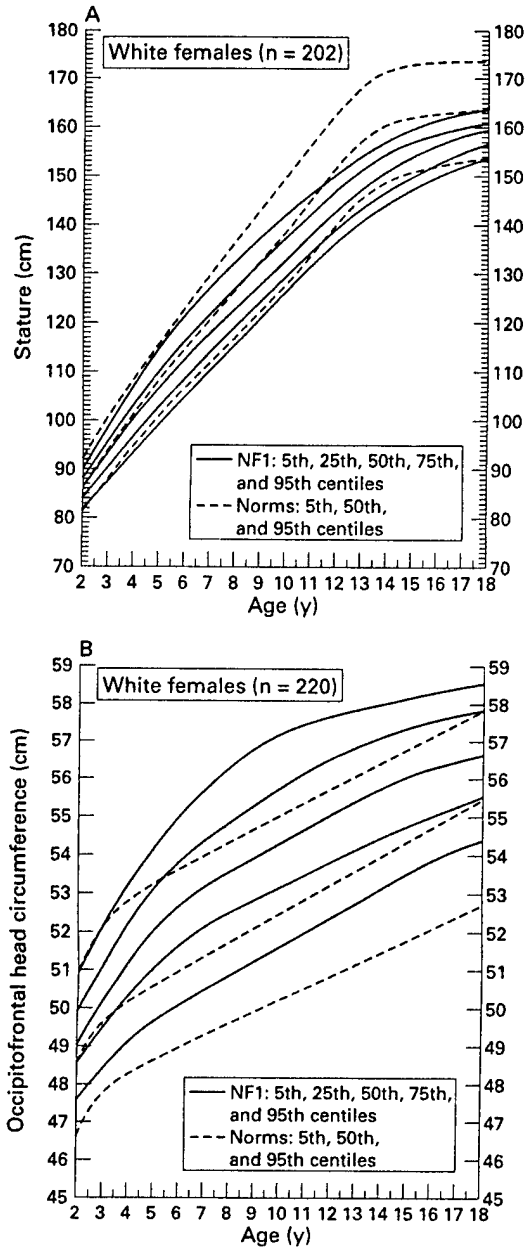

Figure 4 (A) Stature centiles in females 2-18 years. NF1 patient measurements are from the National NF Foundation Database and are denoted by solid lines. Unaffected norms are from the National Center for Health Statistics and the Fels Institute and are denoted by dashed lines. (B) Occipitofrontal circumference centiles in females 2-18 years. NF1 patient measurements are from the National NF Foundation Database and are denoted by solid lines. Unaffected norms are from the National Center for Health Statistics and the Fels Institute and are denoted by dashed lines.

Standardisation for age and sex by z scores is a transformation that allows pooling of measurements across groups that differ in age and sex. This transformation can be applied to measurements for which standard population distributions are approximately normal. The distributions of stature and OFC satisfy this criterion.[12] Thus, a subject's z score closely corresponds to his or her centile rank.

We were concerned that differences between centres would increase the variability of our sample and diminish our ability to analyse the standardised distributions. However, analysis of variance detected no differences for stature or OFC among the contributing centres.

The shifts in the standardised distributions of stature and OFC (figs 1 and 2) confirm that, on average, our NF1 patients are shorter and have bigger heads than standard populations. The shifts are very similar to those in a recent longitudinal study by Carmi *et al.*[20] The population norms were not taken during the same years as our sample, and some were taken 20 or more years earlier. Secular trends suggest that stature and OFC may have increased in the normal population over this time.[16 21-23] If year specific standards were used in our study and the one by Carmi *et al*,[20] the shift in stature may be slightly larger than indicated here and the shift in OFC may be slightly smaller. Ascertainment bias may also affect the distribution shifts. Although short stature and macrocephaly are not among the diagnostic criteria for NF1, these features may have contributed to the patients' referral to the contributing NF clinics.[9] Therefore, the group in this study may be shorter and have bigger heads than a population based sample of children with NF1.

The distributions of standardised stature and OFC are more abruptly peaked than a normal distribution, indicating a relative excess of cases around the mean and in the tails. This variability could result from several factors. (1) The NFDB patient measurements are cross sectional and, therefore, more variable than longitudinal data. (2) Our patient group is geographically heterogeneous. (3) A small proportion of cases may have data entry errors. (4) Ascertainment bias may increase the frequency of outliers. (5) Such distributions might represent composites of more than one normally distributed group with the same mean but different variances.[24]

Riccardi[5] has suggested that short stature and macrocephaly in NF1 are "all or none" phenomena, that is, that two different groups of NF1 patients exist, those with short stature (or macrocephaly) and those without. Under this hypothesis, the distributions should be bimodal, with one mode more than 2 SD outside the normal mean. Our findings are not consistent with this suggestion. The distributions (figs 1 and 2) indicate that stature is reduced to some degree and OFC enlarged to some extent in all NF1 patients.

Our centile curves for stature and OFC (figs 3 and 4) are comparable to those from a recent study of Italian NF1 patients.[25] Minor differences may be partly because of line smoothing techniques and geographical variation. Deviation from these NF1 standards may indicate the effect of a specific disease feature such as optic glioma or hydrocephalus. NF1 specific charts may also provide reassurance that an affected child's growth, although outside the "normal" range on standard paediatric growth charts, is actually normal for a child with NF1. Charts for body mass index and the ratio OFC/stature by age and gender in white NF1 patients are available from http://www.medgen.ubc.ca/friedmanlab.

Patients with hydrocephalus and plexiform neurofibromas of the head were excluded from analyses of OFC, so enlargement of the head in the remaining patients must be the result of

enlargement of the scalp, skull, or brain. In NF1, enlargement of the brain is the probable cause.[5 8] Glial cell proliferation is increased in vitro by sera from NF1 patients, compared to sera from unaffected subjects.[26] Optic or other CNS gliomas are another manifestation of glial cell proliferation. They were observed by MRI in 10% of people in the NFDB affected with NF1. Other studies have observed optic gliomas in 1.5% of 135 and 15% of 217 NF1 patients.[27 28] Glial overgrowth is an important part of NF1 and it may be responsible for macrocephaly in NF1 patients.

Patients with puberty disturbance or bone abnormalities were excluded from analyses of stature. The cause of the stature reduction in the remaining NF1 patients is unknown, but it appears to affect the skeleton proportionately.[5] Data reviewed by Howell *et al*[29] indicate that growth hormone replacement therapy resulted in a moderate increase in stature for NF1 patients with biochemical evidence of growth hormone deficiency. However, growth hormone deficiency was found in only three (2.5%) of 122 children with NF1 in another study.[30] Short stature occurs much more frequently (13%) in the NFDB than can be attributed to such deficiency. Although growth hormone levels were not measured routinely in the NFDB patients, less than 1% are known to have ever had documented growth hormone deficiency.

The findings in this study are consistent with known molecular function of the *NF1* gene/protein. The NF1 protein, neurofibromin, is involved in control of cellular growth and differentiation through the interaction of its GAP related domain with p21ras and tubulin.[31] Neurofibromin is expressed in many different tissues, including the brain, and mutations in the GAP related domain produce hyperactivity of p21ras, which leads to aberrant signalling for cell proliferation.[32] This may contribute to increased glial (astrocyte) cell proliferation and to enlargement of the brain in NF1 patients.[32 33]

The *NF1* homologue in *Drosophila* acts as an activator of the cAMP pathway as well as a negative regulator of Ras.[34] *Drosophila* homozygous for either of two particular *NF1* mutants that lack expression of NF1 protein are 20 to 25% smaller than flies of the parental strain.[35] This growth defect was rescued not only by an *NF1* transgene but also by expression of activated protein kinase A, suggesting protein kinase A functions downstream of or parallel to neurofibromin. Deficiencies in this pathway may contribute to a smaller phenotype in humans as well. Activated protein kinase A is known to stimulate proliferation in some cell types and may normally contribute to body growth.[36 37] Normal stimulation of the protein kinase A pathway also accelerates differentiation and inhibits proliferation of glial (oligodendrocyte) cells.[38 39] Neurofibromin involvement in or between the protein kinase A and p21ras pathways may contribute to the larger heads observed in people diagnosed with NF1.[40] However, our patients with the smallest stature did not also have the largest heads (results not shown).

Short stature and macrocephaly are well recognised clinical features of NF1. This study suggests that these changes in growth affect all NF1 patients and are not limited to particular subgroups. The mechanisms by which mutations of the *NF1* gene produce these phenotypic effects are unknown, but understanding how they do so may provide an important clue to the pathogenesis of more serious manifestations of NF1.

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1 Crowe FW, Schull WJ, Neel JV. *A clinical pathological and genetic study of multiple neurofibromatosis.* Springfield, Illinois: Charles C Thomas, 1956.
2 Littler M, Morton NE. Segregation analysis of peripheral neurofibromatosis (NF1). *J Med Genet* 1990;**27**:307-10.
3 Carey JC, Laub JM, Hall BD. Penetrance and variability in neurofibromatosis: a genetic study of 60 families. *Birth Defects Ser* 1979;**15**:271-81.
4 Huson SM, Harper PS, Compston DA. Von Recklinghausen neurofibromatosis. A clinical and population study in south-east Wales. *Brain* 1988;**111**:1355-81.
5 Huson SM. Neurofibromatosis 1. A clinical and genetic overview. In: Huson SM, Hughes RAC, eds. *The neurofibromatoses. A pathogenic and clinical overview.* London: Chapman and Hall Medical, 1994:160-204.
6 National Institutes of Health Consensus Development Conference. Neurofibromatosis conference statement. *Arch Neurol* 1988;**45**:575-8.
7 Gutmann DH, Aylsworth A, Carey JC, Korf B, Marks J, Pyeritz RE, Rubenstein A, Viskochil D. The diagnostic evaluation and multidisciplinary management of neurofibromatosis 1 and neurofibromatosis 2. *JAMA* 1997;**278**:51-7.
8 Friedman JM, Birch P, Greene C. National Neurofibromatosis Foundation International Database. *Am J Med Genet* 1993;**45**:88-91.
9 Hamill PV, Drizd TA, Johnson CL, Reed RB, Roche AF. NCHS growth curves for children birth-18 years, US 1967-73. *Vital and health statistics.* Series 11, No 165. DHHS Pub No (PHS) 78-1650. Washington: US Government Printing Office, 1977.
10 Horbar JD, Leahy KA. An assessment of data quality in the Vermont-Oxford Trials Network database. *Control Clin Trials* 1995;**16**:51-61.
11 Reynolds-Haertle RA, McBride R. Single vs double data entry in CAST. *Control Clin Trials* 1992;**13**:487-94.
12 SAS Institute. *SAS release 6.12*, Cary, NC: 1996.
13 Hartigan PM. Computation of the dip statistic to test for unimodality. *Appl Stat* 1985;**34**:320-5.
14 Reinsch C. Smoothing by spline functions. *Numerische Mathematik* 1967;**10**:177-83.
15 Kuczmarski RJ, Ogden CL, Grummer-Strawn LM. CDC growth charts: United States. URL: http://www.cdc.gov/nchs/data/ad314.pdf, 2000.
16 Carmi D, Shohat M, Metzker A, Dickerman Z. Growth, puberty, and endocrine functions in patients with sporadic or familial neurofibromatosis type 1: a longitudinal study. *Pediatrics* 1999;**103**:1257-62.
17 Ounsted M, Moar VA, Scott A. Head circumference charts updated. *Arch Dis Child* 1985;**60**:936-9.
18 Roche AF. Secular trends in human growth, maturation, and development. *Monogr Soc Res Child Dev* 1979;**44**:1-120.
19 Zar JH. *Biostatistical analysis.* Upper Saddle River, New Jersey: Prentice Hall, 1999:69.
20 Clementi M, Milani S, Mammi I, Boni S, Monciotti C, Tenconi R. Neurofibromatosis type 1 growth charts. *Am J Med Genet* 1999;**87**:317-23.
21 Caronti B, Buttarelli FR, Giustini S, Calderaro C, Calandriello L, Calvieri S, Palladini G. Serum mitogenic activity on in vitro glial cells in neurofibromatosis type 1. *Brain Res* 1998;**793**:21-8.
22 Huson SM, Harper PS, Compston DA. Von Recklinghausen neurofibromatosis. A clinical and population study in south-east Wales. *Brain* 1988;**111**:1355-81.
23 Lewis RA, Gerson LP, Axelson KA, Riccardi VM, Whitford RP. von Recklinghausen neurofibromatosis. II. Incidence of optic gliomata. *Ophthalmology* 1984;**91**:929-35.
24 Howell SJ, Wilton P, Lindberg A, Shalet SM. Growth hormone replacement and the risk of malignancy in children with neurofibromatosis. *J Pediatr* 1998;**133**:201-5.
25 Cnossen MH, Stam EN, Cooiman LC, Simonsz HJ, Stroink H, Oranje AP, Halley DJ, de Goede-Bolder A, Niermeijer MF, de Muinck Keizer-Schrama SM. Endocrinologic disorders and optic pathway gliomas in children with neurofibromatosis type 1. *Pediatrics* 1997;**100**:667-70.
26 Shen MH, Harper PS, Upadhyaya M. Molecular genetics of neurofibromatosis type 1 (NF1). *J Med Genet* 1996;**33**:2-17.

27 Nordlund M, Gu X, Shipley MT, Ratner N. Neurofibromin is enriched in the endoplasmic reticulum of CNS neurons. *J Neurosci* 1993;**13**:1588-600.
28 Rizvi TA, Akunuru S, de Courten-Myers G, Switzer RC III, Nordlund ML, Ratner N. Region-specific astrogliosis in brains of mice heterozygous for mutations in the neurofibromatosis type 1 (Nf1) tumor suppressor. *Brain Res* 1999; **816**:111-23.
29 Guo HF, The I, Hannan F, Bernards A, Zhong Y. Requirement of Drosophila NF1 for activation of adenylyl cyclase by PACAP38-like neuropeptides. *Science* 1997;**276**:795-8.
30 The I, Hannigan GE, Cowley GS, Reginald S, Zhong Y, Gusella JF, Hariharan IK, Bernards A. Rescue of a Drosophila NF1 mutant phenotype by protein kinase A. *Science* 1997;**276**:791-4.
31 Miyazaki M, Wahid S, Bai L, Namba M. Effects of intracellular cyclic AMP and cyclic GMP levels on DNA synthesis of young-adult rat hepatocytes in primary culture. *Exp Cell Res* 1992;**200**:404-9.
32 Kim HA, DeClue JE, Ratner N. cAMP-dependent protein kinase A is required for Schwann cell growth: interactions between the cAMP and neuregulin/tyrosine kinase pathways. *J Neurosci Res* 1997;**49**:236-47.
33 Raible DW, McMorris FA. Induction of oligodendrocyte differentiation by activators of adenylate cyclase. *Neurosci Res* 1990;**27**:43-6.
34 Raible DW, McMorris FA. Oligodendrocyte differentiation and progenitor cell proliferation are independently regulated by cyclic AMP. *J Neurosci Res* 1993;**34**:287-94.
35 Izawa I, Tamaki N, Saya H. Phosphorylation of neurofibromatosis type 1 gene product (neurofibromin) by cAMP-dependent protein kinase. *FEBS Lett* 1996;**382**:53-9.

Height and head circumference in patients with neurofibromatosis type 1 (NF1). *J. Szudek, P. H. Birch, J. M. Friedman, NNFFID Participants.* Dept. of Medical Genetics, Univ. of British Columbia, Vancouver, B.C., Canada.

NF1 is a progressive disease. Among its many features are short stature and macrocephaly. This study examined the population distribution of height and head circumference (HC) in people with NF1.

The National Neurofibromatosis Foundation International Database (NNFFID) contains clinical information on 2374 NF1 probands. The height and HC of each NNFFID proband were compared to age and sex-specific standards from the Fels Longitudinal Study and norms for Denver and Ohio children. NNFFID data were converted into z-scores above and below the standard means. Patients were grouped by 3-year age intervals, and the mean z-score, and 95% C.I. for height and HC were plotted for each age group and sex.

The HC of NF1 patients is larger than the standard, in both sexes, and in every age category. Mean HC in NF1 patients increases gradually with age in comparison to the standard through at least the first 20 years of life. There were no significant differences between the sexes. Overall, NF1 patient HC distribution was unimodal and symmetrical. The mean HC was 1.00±.07 standard deviations above the standard population mean.

NF1 patients are shorter in both sexes, in most age categories. Mean height decreases with age in comparison to the standard between birth and 15 years. The sharpest decrease is between ages 10 and 15. Although there appear to be differences between male and female trends, these are not statistically significant. Overall, NF1 patients' heights were .59±.07 standard deviations below the standard population mean.

These findings suggest that the heads of NF1 patients grow at a greater than normal rate until the end of puberty and may continue growing longer than in unaffected individuals. Heights in NF1 patients increase at a slower than normal rate from birth to beyond puberty. The shape of the height and HC distributions indicate that these difference in head growth and height affect all NF1 patients to some degree and are not restricted to a subset of NF1 patients.

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Associations of Clinical Features in Neurofibromatosis 1 (NF1)

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Neurofibromatosis 1 (NF1), an autosomal dominant disease, exhibits extreme clinical variability. This variability greatly increases the burden for affected families and impairs our ability to understand the pathogenesis of NF1. Recognition of heterogeneity within a disease may provide important pathogenic insights, therefore we tested clinical data from three large sets of NF1 patients for evidence that certain common features are more likely to occur in some NF1 patients than in others. Clinical information on 4,402 patients with NF1 was obtained from three independent databases. We examined associations between pairs of clinical features in individual affected probands. We also examined associations between the occurrence of individual features in affected relatives. Associations were summarized as odds ratios with 95% confidence intervals. We found associations between several pairs of features in affected probands: intertriginous freckling and Lisch nodules, discrete neurofibromas and plexiform neurofibromas, discrete neurofibromas and Lisch nodules, plexiform neurofibromas and scoliosis, learning disability or mental retardation and seizures. We also found associations between the occurrence of Lisch nodules, macrocephaly, short stature, and learning disability or mental retardation as individual features in parents and children with NF1.

Our observations suggest that, contrary to established belief, some NF1 patients are more likely than others to develop particular manifestations of the disease. Genetic factors appear to determine the development of particular phenotypic features. Genet. Epidemiol. 19:00–00, 2000.

Key words: database; phenotype; proband; familial

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INTRODUCTION

Neurofibromatosis 1 (NF1) is a progressive autosomal dominant disorder affecting approximately 1 in 3,000 people [Crowe et al., 1956; Huson et al., 1989; Littler and Morton, 1990]. Its most frequent features include café-au-lait macules, Lisch nodules, discrete and plexiform neurofibromas, and learning disabilities. Although NF1 has been recognized clinically for more than 100 years [von Recklinghausen, 1882], its natural history is not completely characterized and the pathogenesis is poorly understood. The disease is fully penetrant, but expressivity is variable [Riccardi, 1992]. This variability confounds clinical management and genetic counseling.

The *NF1* locus, identified and sequenced 9 years ago, is the second largest human gene known [Cawthon et al., 1990; Viskochil et al., 1990; Wallace et al., 1990]. Owing to its large size, the lack of clustering of mutation sites and the fact that recurrent mutations are uncommon, molecular genetic testing is not routinely used [Gutmann et al., 1997]. NF1 remains a clinical diagnosis based on the presence of characteristic physical signs or an affected first-degree relative [NIH, 1988; Gutmann et al., 1997]. *NF1* gene mutations have been reported in fewer than 300 patients [NNFF, 1999].

Manifestations of NF1 vary at different times in an individual's life [Moritz and Sneider, 1962; Fitzpatrick et al., 1983; Knight and Reiner, 1983; Riccardi, 1992; Dugoff and Sujanksy, 1996]. Substantial variability also exists among affected members of a single family [Crowe et al., 1956; Zoller et al., 1995]. Nevertheless, there is evidence that related individuals with NF1 are more similar to each other than to unrelated affected individuals. Easton et al. [1993] found evidence of intra-familial correlations in the number of café-au-lait macules and neurofibromas and in the presence or absence of optic gliomas, scoliosis, seizures, and referral for remedial education. There are also families in which an unusual phenotype imparted by an *NF1* mutation appears to "breed true." For example, in families with the Watson syndrome variant of NF1, affected relatives all have features of Watson syndrome rather than typical NF1 [Upadhyaya et al., 1990; Allanson et al., 1991]. In other families, mutations of the *NF1* locus appear to be expressed consistently as multiple café-au-lait spots without other manifestations of NF1 [Abeliovich et al., 1995] or with spinal neurofibromas in multiple generations [Pulst et al., 1991; Poyhonen et al., 1997; Ars et al., 1998].

Recognition of clinical heterogeneity within a disease may provide important pathogenic insights. For example, understanding that NF1 and NF2 are different diseases [Riccardi, 1982] was a seminal contribution. To determine whether clinical heterogeneity exists within NF1 itself, we tested three large clinical datasets for associations between pairs of clinical features in probands. We also tested for genetic determinants of clinical variability by looking for associations of individual clinical features between parents and children with NF1. We found consistent associations among the occurrence of different clinical features in individual patients and between the occurrence of the same feature in relatives. Our observations complement those of Easton et al. [1993] and suggest that, contrary to traditional belief [Bernhart and Halperin, 1990; Riccardi, 1992], some NF1 patients are more likely than others to develop particular manifestations of the disease.

SUBJECTS AND METHODS

Patients and Data Description

All patients included in this analysis were diagnosed with NF1 according to established clinical criteria [NIH, 1988; Gutmann et al., 1997]. The study was performed using clinical data from three independent sets of NF1 patients. At the time of this analysis, the National Neurofibromatosis Foundation International Database (NFDB) [Friedman and Birch, 1997] contained descriptions of 2,509 NF1 probands, 211 affected parents, and 289 of their affected children. Of the NF1 cases 83% are Caucasian, 7% Asian, and 4% African American. The remaining 6% are mostly combinations of these three ethnic groups. The Neurofibromatosis Institute Database (NFID) [Riccardi, 1992] includes standard clinical information on 774 NF1 probands, 132 affected parents, and 189 of their affected children. Of the cases 72% are Caucasian, 14% Hispanic, 13% African American, and 1% Asian. The Manchester NF1 database (MANF1) [McGaughran et al., 1999] includes clinical information on 270 probands, 94 affected parents, and 140 of their affected children. Of the cases 92% are Caucasian, 4% Indian, 2% black, 1% Bangladeshi, and 1% Pakistani. There is no overlap among the patients included in these three databases. Specific *NF1* mutations have been identified by molecular analysis in <1% of these patients.

Statistical Analysis

Twelve of the most common or important clinical features of NF1 were selected for inclusion in this study: intertriginous freckling, discrete cutaneous or subcutaneous neurofibromas (referred to as "discrete neurofibromas"), diffuse or nodular plexiform neurofibromas (referred to as "plexiform neurofibromas"), learning disability or mental retardation, Lisch nodules, scoliosis, tibial or other long bone bowing or pseudarthrosis, optic glioma, macrocephaly, short stature, seizures, and neoplasms (other than neurofibromas or optic glioma). Table I summarizes the prevalence of these 12 features in the three databases.

Most of the features were identified by physical examination. Discrete neurofibromas were coded as "present" if the subject had two or more cutaneous or subcutaneous neurofibromas. Short stature was coded as "present" if the subject's height was ≥2 standard deviations below the age- and gender-matched population mean. Subjects with pseudarthrosis, early or delayed puberty, scoliosis, vertebral dysplasia, or spinal compression were excluded from analyses involving height. Macrocephaly was coded as "present" if the subject's head circumference was ≥2 standard deviations above the age- and gender- matched population mean. Subjects with plexiform neurofibroma of the head, early or delayed puberty, or hydrocephalus were excluded from analyses involving head circumference. Lisch nodules were diagnosed or excluded by a slit-lamp examination. The presence or absence of optic glioma was determined by cranial magnetic resonance imaging or computed tomography examination. Only patients who had definite presence or absence of a feature were considered in comparisons involving that feature.

Pair-wise combinations of the presence or absence of each feature were analyzed in probands by 2x2 tables using SAS [SAS Institute, 1996]. The prevalence of many features of NF1 increases with age [Riccardi, 1992; Cnossen et al., 1998]. Two

TABLE I. Prevalence of Clinical Features of NF1 in Probands and Affected Relatives (these frequencies vary greatly by age, but all subjects are included in this table to provide an overview of the data sets in our studies)

	NFDB				NFID				MANF1			
	Probands		Affected relatives		Probands		Affected relatives		Probands		Affected relatives	
Clinical feature	%	(*n*)	%	(*n*)	%	(*n*)	%	(*n*)	%	(*n*)	%	(*n*)
Freckling	82.9	(2420)	77.2	(452)	75.8	662)	75.0	(148)	90.8	(228)	81.8	(132)
Discrete NFs	52.5	(2499)	51.0	(467)	51.8	(713)	45.8	(168)	86.0	(242)	54.9	(122)
Plexiform NFs	25.8	(2490)	15.2	(467)	41.5	(743)	25.3	(178)	19.3	(270)	15.2	(171)
Lisch nodules	55.9	(1837)	63.4	(339)	83.0	(395)	89.1	(101)	70.1	(174)	62.1	(66)
Optic glioma	25.0	(1000)	16.8	(125)	21.3	(400)	11.7	(77)	9.5	(190)	12.9	(70)
Seizures	6.8	(2509)	4.3	(470)	5.9	(732)	3.8	(185)	3.0	(237)	9.4	(149)
LD/MR	47.2	(1899)	52.1	(355)	51.1	(587)	48.6	(142)	24.1	(187)	30.0	(100)
Pseudarthrosis	5.2	(2497)	3.9	(462)	4.0	(756)	2.1	(189)	2.1	(243)	2.7	(149)
Scoliosis	25.6	(2498)	14.0	(463)	25.0	(645)	23.1	(156)	14.6	(246)	14.7	(150)
Macrocephaly	20.1	(1553)	17.6	(301)	30.8	(598)	25.5	(137)	24.2	(186)	19.1	(115)
Short stature	12.6	(1903)	20.1	(353)	7.3	(605)	4.0	(124)	34.3	(134)	56.3	(80)
Neoplasms	6.6	(2509)	3.8	(470)	10.7	(774)	9.1	(208)	6.5	(230)	5.8	(137)

NFs, neurofibromas; LD/MR, learning disability or mental retardation.

features that both increase with age may show a strong association because older patients are likely to have both features and younger patients are likely to have neither. Therefore, patients from each database were stratified into 5-year age groups to reduce confounding by age. Patients were also stratified by gender, but not by race because the number of non-Caucasians is sparse. The method of Mantel and Haenszel [1959] was used to estimate the odds ratio with 95% confidence intervals over the age and gender strata. We performed the analyses in three independent datasets (NFDB, NFID, and MANF1) because we expected to observe many associations that reached nominal statistical significance by chance as a result of multiple comparisons. Odds ratios with 95% confidence intervals that excluded 1.0 in at least two of the three databases were considered unlikely to be owing to chance alone. The Breslow-Day method [SAS Institute, 1996] was used to test each triad of odds ratios for homogeneity between the three databases. Triads with P-values >0.05 were considered homogeneous. The method of Mantel and Haenszel [1959] was used to estimate the summary odds ratio with 95% confidence intervals for all three databases together.

The second analysis included affected relatives. A feature that increases with age may show a strong intra-familial association because the ages of sibs within a family are usually similar. Therefore, we limited our analysis to parents and children, who usually differ in age by at least 20 years. For each of the 12 features, a 2×2 table was used to compare the frequency of a given feature in NF1 children of NF1 parents who had the feature to the frequency in children of parents who lacked the feature. Each individual was counted only once. Twelve contingency tables were generated separately in each database. Odds ratios with 95% confidence intervals were calculated for contingency tables without blank cells. The Breslow-Day method [SAS Institute, 1996] was used to test for homogeneity, and the Mantel-Haenszel method [1959] was used to estimate summary odds ratios.

RESULTS

Associations Between Features in Individual NF1 Probands

Pair-wise associations between each of the 12 clinical features were tested in 2,509 NF1 probands from the NFDB, 774 NF1 probands from the NFID, and 270 NF1 probands from the MANF1 database (Table II). In the NFDB, an odds ratio of 1.0 was excluded from the 95% confidence limits for 26 of 66 associations tested. There were 23 nominally significant positive associations and three nominally significant inverse associations. In the NFID, which contains fewer than one third as many cases as the NFDB, an odds ratio of 1.0 was excluded from the 95% confidence limits for 13 of 66 associations tested. Ten of these nominally significant associations were positive and three were negative. In the MANF1, which is approximately one ninth as large as the NFDB, an odds ratio of 1.0 was excluded from the 95% confidence limits for five of 55 associations tested. All these were positive. Odds ratios could not be calculated in the remaining 11 associations owing to blank cells in the contingency tables. Overall, six of 66 tested associations between pairs of features are statistically significant and in the same direction in at least two of the databases (Table II). Four of these six associations are statistically homogeneous between the three databases. One statistically significant inverse association was observed in at least two independent databases. The associations are in Table II as odds ratios for each database and as summary odds ratios for all three databases together.

Associations of Features Between Affected Parents and Children

Table III summarizes the associations for occurrence of the 12 features between: 211 NF1 parents and 289 of their NF1 children from the NFDB, 132 NF1 parents and 189 of their NF1 children from the NFID, and 94 NF1 parents and 140 of their NF1 children from the MANF1. The associations are expressed as odds ratios for each database and as summary odds ratios for all three databases together. Odds ratios could not be calculated for one association in the NFBD, three associations in the NFID, and two associations in the MANF1 owing to blank cells in contingency tables. A summary odds ratio of one was excluded from the 95% confidence limits in four of the 12 associations between parents and children. Three of these four associations are statistically homogeneous between the three databases. No significant negative associations were observed.

DISCUSSION

Associations Between Features in Individual NF1 Probands

The large number of cases in these three databases enables us to find significant associations between several common features of NF1 (Table II). The concordance between the findings in the three independent databases is remarkable. Approximately three ($P = 0.05$ multiplied by 66) nominally statistically significant associations were expected by chance in each database, and one would expect chance associations to differ in the NFDB, NFID, and MANF1. The reproducibility of our results suggests that these associations are probably not owing to chance alone.

The positive associations observed may reflect shared pathogenic mechanisms underlying the associated features. For example, NF1 probands with seizures may be

TABLE II. Odds Ratios with 95% Confidence Limits for Associations of Features Among Age and Gender-Stratified Probands with NF1

Associated features		NFDB	NFID	MANF1	Homogeneity (*P*)	Summary odds ratio
Freckling	Lisch nodules	1.8 (1.3–2.4)	1.2 (0.6–2.5)	3.2 (1.2–9.0)	0.85	1.7 (1.3–2.3)
Discrete NFs	Plexiform NFs	2.9 (2.3–3.6)	1.9 (1.2–2.9)	2.1 (0.7–6.4)	0.11	2.6 (2.1–3.1)
Discrete NFs	Lisch nodules	1.6 (1.3–2.0)	2.6 (1.3–5.4)	2.1 (0.9–4.7)	0.04	1.7 (1.3–2.1)
Discrete NFs	Pseudarthrosis	0.5 (0.3–0.9)	0.3 (0.1–0.9)		0.04	0.5 (0.4–0.8)
Plexiform NFs	Scoliosis	1.5 (1.2–1.8)	1.8 (1.2–2.6)	1.6 (0.7–3.6)	0.72	1.6 (1.3–1.9)
Seizures	LD/MR	2.2 (1.5–3.1)	3.6 (1.6–7.9)	5.7 (1.3–24.5)	0.18	2.5 (1.8–3.4)

Statistically significant associations are shaded.

Odds ratios with 95% confidence limits could not be determined for comparisons in which contingency tables contained empty cells. The corresponding cells in Table II are blank.

NFs, neurofibromas; LD/MR, learning disability or mental retardation.

TABLE III. Odds Ratio with 95% Confidence Limits for Associations of Features Between Parents and Children with NF1

Feature	NFDB	NFID	MANF1	Homogeneity (*P*)	Summary odds ratio
Freckling	1.9 (0.8–4.7)	0.8 (0.2–3)	1.7 (0.4–7.4)	0.52	1.5 (0.8–2.8)
Discrete NFs	1.8 (0.9–3.9)	3.4 (0.7–16.1)	0.5 (0.1–2)	0.13	1.6 (0.9–2.9)
Plexiform NFs	0.8 (0.4–1.9)	0.6 (0.3–1.4)	1.7 (0.6–5.3)	0.35	0.9 (0.5–1.4)
Lisch nodules	8.5 (3.3–22.3)	5.8 (0.3–100)	10.5 (1.8–61.2)	0.94	8.7 (4.2–18.1)
Optic glioma		2.0 (0.3–12.7)	27.3 (1.9–395)	0.17	3.6 (1.1–12.3)
Seizures	1.2 (0.1–9.3)	7.1 (0.6–84.6)		0.38	1.7 (0.4–7.5)
LD/MR	1.3 (0.8–2.2)	5.8 (2–16.6)	16.8 (1.8–160)	0.004	2.0 (1.3–3.1)
Pseudarthrosis	5.1 (0.5–49.3)				1.8 (0.2–14.3)
Scoliosis	1.7 (0.7–3.8)	1.6 (0.6–4.1)	1.3 (0.3–6.6)	0.96	1.6 (0.9–2.8)
Macrocephaly	8.2 (2.9–22.7)	2.0 (0.8–5.2)	2.6 (0.5–12.4)	0.12	3.5 (1.9–6.2)
Short stature	5.9 (2.3–14.8)		2.3 (0.6–9.2)	0.47	4.2 (2.0–8.6)
Neoplasms	5.0 (1.0–26.4)		25.5 (1.3–485)	0.001	1.3 (0.4–3.9)

Statistically significant associations are shaded.
Odds ratios with 95% confidence limits could not be determined for comparisons in which contingency tables contained empty cells. The corresponding cells in Table III are blank.
NFs, neurofibromas; LD/MR, learning disability or mental retardation.

more likely also to have learning disabilities or mental retardation than patients without seizures (Table II) because the effect of the *NF1* mutation on brain development is greater in patients who have seizures.

The association observed between the occurrence of plexiform and discrete neurofibromas (Table II) is consistent with the histopathological similarity between these lesions [Harkin and Reed, 1969; Burger and Scheithauer, 1994]. In addition, both kinds of neurofibromas are associated with acquired loss or mutation of the normal *NF1* allele in at least some cases [Serra et al., 1997; Sawada et al., 1996]. NF1 patients who develop plexiform neurofibromas usually do so during childhood [Riccardi, 1992]. In contrast, discrete neurofibromas are uncommon in young children but are almost universally present among adults with NF1. The association we observed is much stronger in younger than in older NF1 patients. The odds ratio was 6.9 among patients younger than 5 years old, 3.1 among those 5 to 9, but only 1.3 among those older than 40. This raises the interesting possibility that NF1 patients with plexiform neurofibromas develop discrete neurofibromas earlier than patients without plexiform lesions.

The associations between Lisch nodules and both discrete neurofibromas and intertriginous freckling (Table II) were previously reported [Pietruschka, 1961; Zehavi et al., 1986], but the responsible mechanism is unknown. Lisch nodules [Perry and Font, 1982] and freckles [Fitzpatrick, 1981] are derived from cells of melanocytic origin, and all three lesions involve cells derived from the embryonic neural crest [Weston et al., 1981]. This is consistent with the suggestion that NF1 is a neurocristopathy [Huson and Hughes, 1994] but does not explain why other neural crest-derived tissues, such as the sympathetic ganglia, thyroid C-cells, and parathyroids, are rarely involved in NF1. Moreover, many features of NF1, such as learning disabilities, dysplastic scoliosis, and tibial pseudarthrosis, do not appear to be abnormalities of neural-crest derived tissues.

Although plexiform neurofibromas growing near the spine can cause abnormal

curvature and result in scoliosis, the association we observed between plexiform neurofibromas and scoliosis does not lose significance when patients with plexiform neurofibromas of the trunk are excluded. Furthermore, two different forms of scoliosis may occur in NF1 patients: a dystrophic form that occurs within the first decade of life and is often severe and rapidly progressive and a milder form that occurs later and resembles common adolescent scoliosis [Riccardi, 1992]. The association we observed involves only early-onset scoliosis. The pathogenic basis for this association and for the association we observed between the absence of pseudarthrosis or bowing and discrete neurofibromas is obscure.

Most of the associations observed among probands in this study are moderate in strength—positive associations generally have odds ratios in the range of 2.0–3.0 and negative associations have odds ratios in the range of 0.3–0.5 (Table II). Such pairwise associations are not strong enough to be useful clinically for predictive classification of patients. Nevertheless, our observation of similar associations in three independent databases strongly suggests that common disease features do not occur entirely at random in NF1 and that some patients are more likely than others to develop particular features. This interpretation contrasts with the generally held view that any NF1 patient may develop any manifestation of the disease [Bernhart and Halperin, 1990; Riccardi, 1992]. Most of the associations we observed have never been noted before. Their identification is an important step toward understanding the pathogenesis of NF1.

Associations of Features Between Affected Parents and Children

Our observations in probands suggest that shared pathogenic mechanisms underlie several common features of NF1. If genetic factors influence these pathogenic mechanisms, one would expect familial aggregation of such features to occur. Therefore, we tested for associations between the occurrence of the 12 features among affected relatives.

The ages of sibs within a family are usually similar, and an association may be noted because older sib-pairs are more likely to both have a feature and younger sib-pairs to both lack a feature that increases in prevalence with age. Parents and children usually differ in age by at least 20 years, so significant associations between the occurrence of a feature in a parent and child are unlikely to be inflated by age confounding. Owing to this age difference, we expect the odds ratios from parent-child comparisons to yield conservative estimates of intra-familial associations. Consequently, we limited our analysis to affected parents and children.

Several strong associations were found by comparing the presence or absence of the 12 features between affected parents and children (Table III). The summary odds ratios for Lisch nodules, optic glioma, macrocephaly, and short stature were significant and homogeneous among the three databases. The summary odds ratio for learning disability or mental retardation was significant but was not homogeneous between the three databases. This may be owing to differences among centers in how the feature is diagnosed. These associations are probably not owing to ascertainment bias. All subjects were assessed in specialized NF clinics, and the family was excluded from a particular analysis if the presence or absence of the feature in question was not known in both the affected parent and child.

No negative associations were found between affected parents and children. This is consistent with our hypothesis that affected relatives have a more similar NF1

phenotype than unrelated people. The absence of negative associations also supports the statistical validity of our observations. One would expect to observe negative, as well as positive, associations by chance.

We observed familial associations for the occurrence of Lisch nodules, macrocephaly, short stature, and learning disability or mental retardation. In a previous study, Easton et al. [1993] examined 175 individuals with NF1 and found evidence of intrafamilial correlations in the number of café-au-lait macules and neurofibromas and in the presence or absence of optic gliomas, scoliosis, seizures, and referral for remedial education. Easton et al. observed no correlations for head circumference or plexiform neurofibromas. Furthermore, phenotypic similarity of these NF1 features was found to decrease with decreasing genetic similarity—a trend not examined here. Unlike our study, the results of Easton et al. rely heavily on data from six pairs of monozygotic twins. Nevertheless, the studies are both consistent with genetic factors influencing the phenotypic expression of *NF1* mutations in patients with NF1.

The strong phenotypic similarity among relatives may be evidence of an *NF1* allele-phenotype correlation. Although generally not striking in NF1, phenotypic modification by the nature of the mutant allele has been demonstrated in large deletions of the *NF1* gene, which tend to result in a severe phenotype [Tonsgard et al., 1997]. Other genetic factors that might influence the phenotype in NF1 patients include variants of the normal *NF1* allele and "modifying genes" at other loci.

First-degree relatives share half of their DNA sequences at other loci. Similarities at these other loci may contribute to the phenotypic similarities observed in families with NF1. Our findings complement those of Easton et al. [1993] and are consistent with their hypothesis hat modifying genes influence the NF1 phenotype. The NF1 protein neurofibromin is known to interact with many other proteins, including tubulin [Bollag et al., 1993], kinases [Marchuk et al., 1991], and *Ras* [Buchberg et al., 1990; Xu et al., 1990]. Functional variants of these proteins might also influence the NF1 phenotype.

Our studies demonstrate that, although the NF1 phenotype is highly variable, some patients are more likely than others to develop certain disease features. In addition, we show that genetic factors may determine the particular phenotypic features that develop in many cases. Further clinical, epidemiological, and molecular studies are necessary to elucidate the pathogenesis of this complex disease fully, but our investigations provide hope that some serious complications of NF1 can be predicted or prevented.

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REFERENCES

Abeliovich D, Gelman-Kohan Z, Silverstein S, et al. 1995. Familial cafe au lait spots: a variant of neurofibromatosis type 1. J Med Genet 32:985–6.

Allanson JE, Upadhyaya M, Watson GH, et al. 1991. Watson syndrome: is it a subtype of type 1 neurofibromatosis? J Med Genet 28:752–6.

Ars E, Kruyer H, Gaona A, Casquero P, et al. 1998. A clinical variant of neurofibromatosis type 1: familial spinal neurofibromatosis with a frameshift mutation in the NF1 gene. Am J Hum Genet 62:834–41.

Bernhart B, Halperin JC. 1990. Genetic counseling for neurofibromatosis. In: Rubenstein AE, Korf BR, editors. Neurofibromatosis : a handbook for patients, families, and health-care professionals. New York: Thieme Medical Publishers. p 201–8.

Bollag G, McCormick F, Clark R. 1993. Characterization of full-length neurofibromin: tubulin inhibits Ras GAP activity. EMBO J 12:1923–7.

Buchberg AM, Cleveland LS, Jenkins NA, Copeland NG. 1990. Sequence homology shared by neurofibromatosis type-1 gene and IRA-1 and IRA-2 negative regulators of the RAS cyclic AMP pathway. Nature 347:291–4.

Burger PC, Scheithauer BW. 1994. Tumors of the central nervous system. Atlas of tumor pathology. Third Series, Fascicle 10. Washington, DC: Armed Forces Institute of Pathology.

Cawthon RM, Weiss R, Xu GF, et al. 1990. A major segment of the neurofibromatosis type 1 gene: cDNA sequence, genomic structure, and point mutations. Cell 2:193–201. [Published erratum appears in Cell 62(3).]

Cnossen MH, de Goede-Bolder A, van den Broek KM, et al. 1998. A prospective 10 year follow up study of patients with neurofibromatosis type 1. Arch Dis Child 78:408–12.

Crowe FW, Schull WJ, Neel JV. 1956. A clinical pathological and genetic study of multiple neurofibromatosis. Springfield, IL: Charles C Thomas.

Dugoff L, Sujansky E. 1996. Neurofibromatosis type 1 and pregnancy. Am J Med Genet 66:7–10.

Easton DF, Ponder MA, Huson SM, Ponder BA. 1993. An analysis of variation in expression of neurofibromatosis type 1 (NF1): evidence for modifying genes. Am J Hum Genet 53:305–13.

Fitzpatrick JE, McDermott M, May D, Hofeldt FD. 1983. Eruptive neurofibromatosis associated with anorexia nervosa. Arch Dermatol 119:1019–21.

Fitzpatrick TB. 1981. Melanin synthesis pathways in the pathogenesis of neurofibromatosis. Adv Neurol 29:209–11.

Friedman JM, Birch PH. 1997. Type 1 neurofibromatosis: a descriptive analysis of the disorder in 1,728 patients. Am J Med Genet 70:138–43.

Gutmann DH, Aylsworth A, Carey JC, et al. 1997. The diagnostic evaluation and multidisciplinary management of neurofibromatosis 1 and neurofibromatosis 2. JAMA 278:51–7.

Harkin JC, Reed RJ. 1969. Tumors of the peripheral nervous system. Atlas of tumor pathology. Second Series, Fascicle 3. Washington, DC: Armed Forces Institute of Pathology.

Huson SM, Compston DAS, Clark P, Harper PS. 1989. A genetic study of von Recklinghausen neurofibromatosis in south east Wales—prevalence, fitness, mutation rate and effect of parental transmission on severity. J Med Genet 26:704–11.

Huson SM, Hughes RAC, editors. 1994. The neurofibromatoses: a pathogenetic and clinical overview. New York: Chapman & Hall.

Knight PJ, Reiner CB. 1983. Superficial lumps in children: what, when, and why? Pediatrics 72:147–53.

Littler M, Morton NE. 1990. Segregation analysis of peripheral neurofibromatosis (NF1). J Med Genet 27:307–10.

Mantel N, Haenszel W. 1959. Statistical aspects of the analysis of data from retrospective studies of disease. J Natl Cancer Inst 22:719–48.

Marchuk DA, Saulino AM, Tavakkol R, et al. 1991. cDNA cloning of the type 1 neurofibromatosis gene: complete sequence of the NF1 gene product. Genomics 11:931–40.

McGaughran JM, Harris DI, Donnai D, et al. 1999. A clinical study of type 1 neurofibromatosis in north west England. J Med Genet 36:197–203.

Moritz HC, Sneider E. 1962. Von Recklinghausen's disease exaggerated during pregnancy: case report. Harper Hosp Bull 20:79–82.

National Institutes of Health Consensus Development Conference. 1988. Neurofibromatosis Conference Statement. Arch Neurol 45:575–8.

NNFF International NF1 Genetic Mutation Analysis Consortium. 1999. Available from URL:http://www.nf.org/nf1gene/nf1gene.mutdata.summary.html

Perry HD, Font RL. 1982. Iris nodules in von Recklinghausen's neurofibromatosis. Electron microscopic confirmation of their melanocytic origin. Arch Ophthalmol 100:1635–40.

Pietruschka G. 1961. Zur Symptomatic der Neurofibromatosis multiplex nach von Recklinghausen im Bereich des Sehorgans. Med Bild 4:8–11.

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Poyhonen M, Leisti EL, Kytola S, Leisti J. 1997. Hereditary spinal neurofibromatosis: a rare form of NF1? J Med Genet 34:184–7.

Pulst SM, Riccardi VM, Fain P, Korenberg JR. 1991. Familial spinal neurofibromatosis: clinical and DNA linkage analysis. Neurology 41:1923–7.

Riccardi VM. 1982. Neurofibromatosis: clinical heterogeneity. Curr Probl Cancer 7:1—34.

Riccardi VM. 1992. Neurofibromatosis: phenotype, natural history and pathogenesis. 2nd ed. Baltimore: Johns Hopkins University Press.

SAS Institute. 1996. SAS release 6.12, Cary, NC.

Sawada S, Florell S, Purandare SM, et al. 1996. Identification of NF1 mutations in both alleles of a dermal neurofibroma. Nat Genet 14:110–2.

Serra E, Puig S, Otero D, et al. 1997. Confirmation of a double-hit model for the NF1 gene in benign neurofibromas. Am J Hum Genet 61:512–9.

Tonsgard JH, Yelavarthi KK, Cushner S, et al. 1997. Do NF1 gene deletions result in a characteristic phenotype? Am J Med Genet 73:80–6.

Upadhyaya M, Sarfarazi M, Huson S, et al. 1990. Linkage of Watson syndrome to chromosome 17 markers. J Med Genet 27:209.

Viskochil D, Buchberg AM, Xu G, et al. 1990. Deletions and a translocation interrupt a cloned gene at the neurofibromatosis type 1 locus. Cell 62:187–92.

von Recklinghausen F. 1882. Uber die Multipen Fibrome der Haut und ihre Beziehung zu Multipen Neuromen. August Hirschwald, Berlin.

Wallace MR, Marchuk DA, Andersen LB, et al. 1990. Type 1 neurofibromatosis gene: identification of a large transcript disrupted in three NF1 patients. Science 249:181–6. Published erratum appears in Science 1990;250:1749.

Weston JA. 1981. The regulation of normal and abnormal neural crest cell development. Adv Neurol 29:77–95.

Xu GF, O'Connell P, Viskochil D, et al. 1990. The neurofibromatosis type 1 gene encodes a protein related to GAP. Cell 62:599–608.

Zehavi C, Romano A, Goodman RM. 1986. Iris (Lisch) nodules in neurofibromatosis. Clin Genet 29:51–5.

Zoller M, Rembeck B, Akesson HO, Angervall L. 1995. Life expectancy, mortality and prognostic factors in neurofibromatosis type 1. A twelve-year follow-up of an epidemiological study in Goteborg, Sweden. Acta Dermatol Venereol 75:136–40.

Associations of clinical features in children with neurofibromatosis type 1 (NF1). *J. Szudek*[1], *V.M. Riccardi*[2] *and J.M. Friedman*[1]. [1]University of British Columbia, Canada. [2]Medical Consumers, California.

Most NF1 patients never develop severe complications of the disease, but the variable expressivity of NF1 prevents prediction of which patients will develop such complications. The NNFF International Database and NF Institute Database are independent and contain clinical information on 2098 and 814 NF1 probands, respectively. We used these data to test for associations between the occurrence of various phenotypic features in NF1 probands.

Many manifestations of NF1 increase in frequency with increasing age, so we restricted this analysis to children under 10 years old. Thirty-three features from the NNFF International Database were tested in a pair-wise fashion. The analysis was repeated for the NF Institute Database. In order to minimize chance associations that can result from multiple comparisons, only associations found to be significant ($p<.005$ by Fisher's exact test) in both databases were analyzed further. Combined odds ratios (OR) and 95% confidence intervals (CI) were calculated by the method of Woolf (*Ann Hum Genet 19*:251, 1955).

Several features were found to be associated. Plexiform neurofibromas occurred more often than expected in children with NF1 who have the following clinical features compared to children who lack the feature: both subcutaneous and cutaneous neurofibromas (OR = 8.7, CI 4.1-18.2); only subcutaneous neurofibromas (OR = 3.2, CI 1.9-5.4); only cutaneous neurofibromas (OR = 3.1, CI ,1.8-5.5); hypertension (OR = 12.2, CI 3.5-42.5). Neoplasms other than neurofibromas or optic gliomas were found more often in children who have either subcutaneous or cutaneous neurofibromas than in children without neurofibromas (OR=2.4, CI 1.3-4.4).

Our findings suggest that fundamental pathophysiological differences exist between NF1 patients who develop neurofibromas in childhood and those who do not.

We are grateful to the NNFF International Database Participants who contributed the data analyzed in the study.

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Letter to the Editor

Growth Charts for Young Children With Neurofibromatosis 1 (NF1)

Letter To the Editor:

Short stature and macrocephaly are more common in children with neurofibromatosis 1 (NF1) than in the general population. The cause of these growth alterations is unknown in most cases, but monitoring growth in affected children assists in detection of complications such as optic glioma and hydrocephalus. Clementi et al. [1999] developed growth charts for NF1 patients 2 to 18 years old. We have made similar charts from cross-sectional data obtained from the National NF Foundation International Database (NFDB). These are available from http://mendel.medgen.ubc.ca/friedmanlab. We also developed standard growth charts for total body length (TBL), weight, and occipitofrontal head circumference (OFC) in NF1 patients between 3 and 36 months old.

TBL, weight, and OFC measurements of 336 Caucasian NF1 patients between 3 and 36 months old were obtained from 19 centers contributing data to the NFDB [Friedman et al., 1993]. Patients with clinical findings that could affect measurements were excluded. Analysis of variance was used to determine if significant differences exist among age- and sex-standardized measurements from major contributing centers.

Standard population norms were obtained from studies by the National Center for Health Statistics and the Fels Institute [Hamill et al., 1977; Najjar and Rowland, 1987]. NF1 patients were divided into sex and age groups whose medians correspond to those of the charts for population norms. Centiles were plotted and smoothed using SAS (SAS Institute, Cary, NC, 1996) by producing a cubic spline that minimizes a linear combination of the sum of squares of the residuals of fit and the integral of the square of the second derivative [Reinsch, 1967].

TBL, weight, and OFC centiles by age are shown in Figures 1a–2c. Median TBL is 1–2 cm lower, weight is 0.5–1 kg lower, and OFC is 1–2 cm greater in NF1 patients than in the standard pediatric growth charts. We were concerned that geographic differences [Meredith, 1971] and lack of consistent methodology among centers might increase the variability of our sample and diminish the usefulness of these growth charts. However, ANOVA for heterogeneity among major contributing centers of the NFDB revealed no heterogeneity for TBL ($P = 0.39$) and OFC ($P = 0.31$) in these children.

The contributors to the NFDB are specialized NF1 clinics, and the patients that visit them are likely to be more severely affected than NF1 patients in general. However, TBL, weight, and OFC are not among the cardinal features by which NF1 is diagnosed [NIH, 1988], so it is unlikely that the children we studied differ greatly with respect to these measurements from the NF1 population as a whole.

Charts from standard populations provide a useful reference to follow growth in children. However, once the diagnosis of NF1 has been made charts specific to NF1 are more useful for detection of deviation from the expected growth pattern for that child. Deviation of a child's growth from the NF1 standards may indicate the effect of a specific disease feature such as optic glioma or hydrocephalus. NF1-specific charts may also provide reassurance to families and their physicians that an affected child's growth, although outside the "normal" range on standard pediatric growth charts, is actually normal for a child with NF1. The charts presented here provide an appropriate standard for monitoring growth in young children with NF1.

REFERENCES

Clementi M, Milani S, Mammi I, Boni S, Monciotti C, Tenconi R. 1999. Neurofibromatosis type 1 growth charts. Am J Med Genet 87: 317–323.

Friedman JM, Birch P, Greene C. 1993. National Neurofibromatosis Foundation International Database. Am J Med Genet 45:88–91.

Hamill PV, Drizd TA, Johnson DL, Reed RB, Roche AF. 1977. NCHS growth curves for children birth–18 years. U.S. 1967–73. Vital and health statistics. Series 11, No. 165. DHHS Pub. No. (PHS) 78-1650. Washington: U.S. Government Printing Office.

Meredith HV. 1971. Human head circumference from birth to early adult

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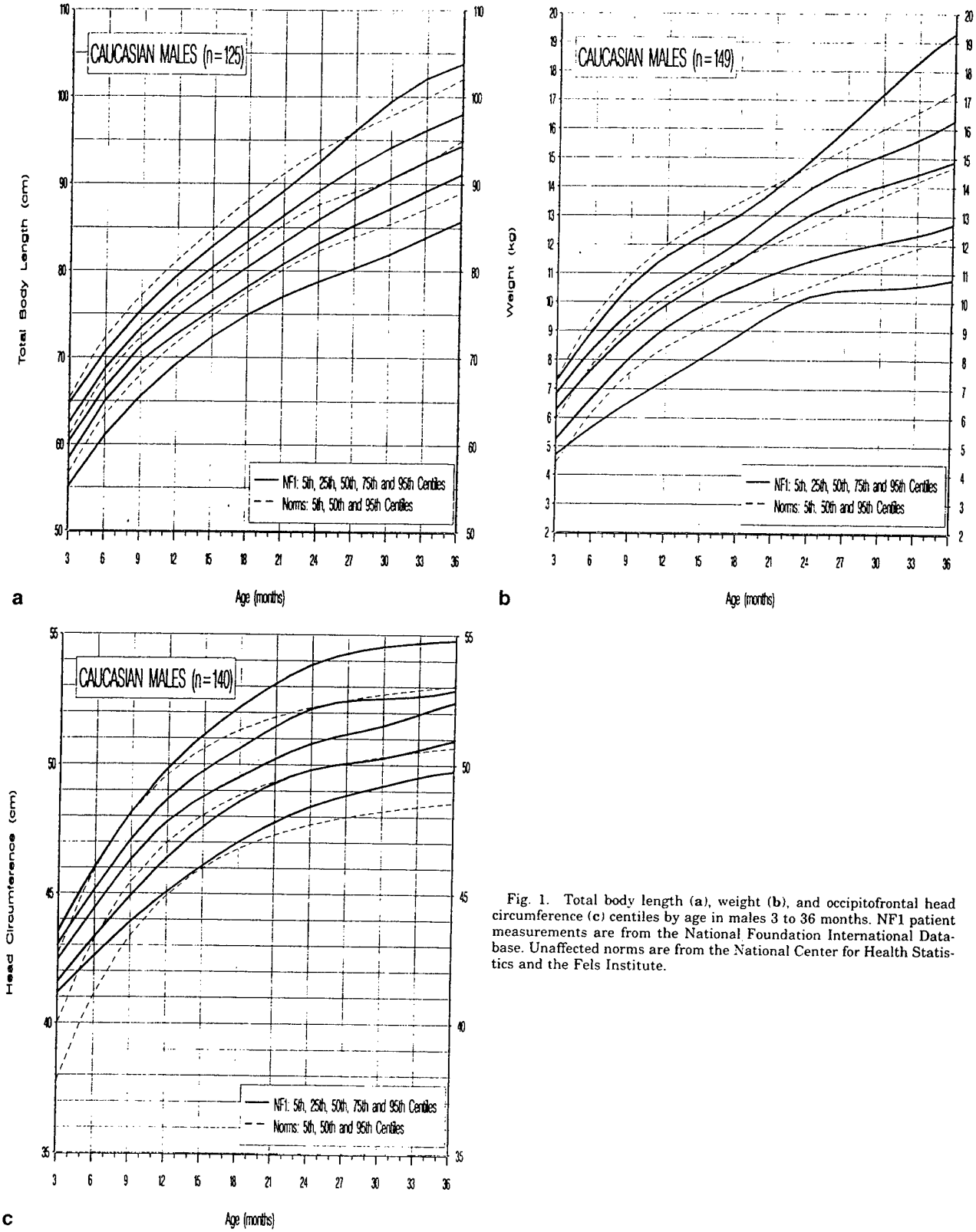

Fig. 1. Total body length (**a**), weight (**b**), and occipitofrontal head circumference (**c**) centiles by age in males 3 to 36 months. NF1 patient measurements are from the National Foundation International Database. Unaffected norms are from the National Center for Health Statistics and the Fels Institute.

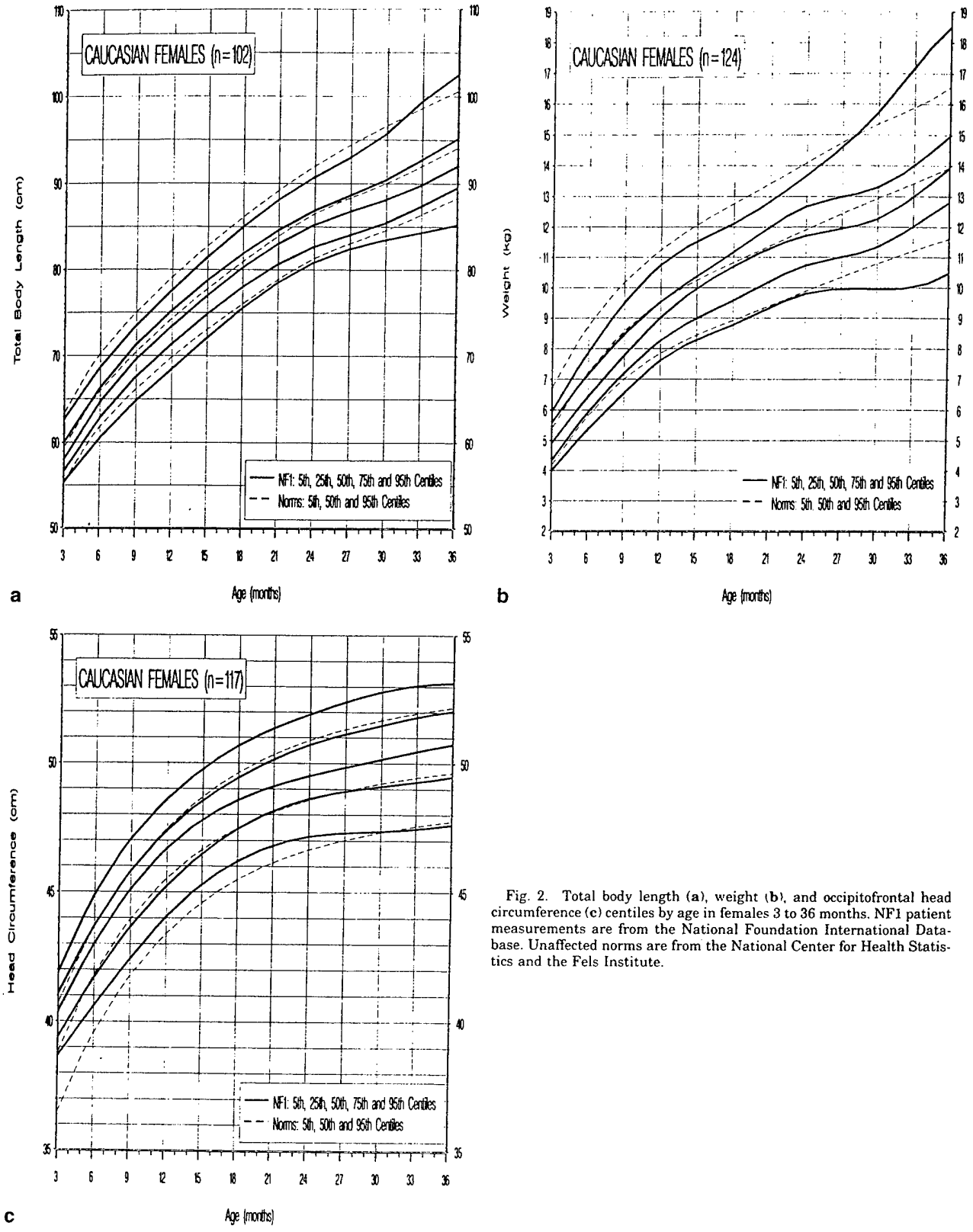

Fig. 2. Total body length (a), weight (b), and occipitofrontal head circumference (c) centiles by age in females 3 to 36 months. NF1 patient measurements are from the National Foundation International Database. Unaffected norms are from the National Center for Health Statistics and the Fels Institute.

hood: racial, regional, and sex comparisons. Adv Child Dev Behav 6: 153–238.

Najjar MF, Rowland M. 1987. Anthropometric reference data and prevalence of overweight, United States. 1976–80. Vital and Health Statistics. Series 11, No. 238. DHHS Pub. No. (PHS) 87 1688. Public Health Service. Washington: U.S. Government Printing Office.

NIH (National Institutes of Health Consensus Development Conference). 1988. Neurofibromatosis Conference Statement. Arch Neurol 45:575–578.

Reinsch C. 1967. Smoothing by spline functions. [illegible] 10:177–183.

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The effect of parental age on the occurrence of Neurofibromatosis 1. *J. Tzenova[1], H. Joe[2], V.M. Riccardi[3], J.M. Friedman[1].* 1) Department of Medical Genetics; 2) Department of Statistics, University of British Columbia, Vancouver, BC, Canada; 3) The Neurofibromatosis Institute, La Crescenta, CA.

New mutations account for half of all patients with neurofibromatosis 1 (NF1), and about 80% of new mutations occur in the paternally-inherited allele. The exception is large deletions, which are predominantly maternal in origin. Typical NF1 may also occasionally result from somatic mutations. Previous studies of paternal age among patients with sporadic NF1 have been inconclusive. We postulated that failure to find a paternal age effect in these studies may have resulted from inclusion of patients with large deletions and somatic mutations, for whom no paternal age effect would be expected.

In order to test this possibility, we used data collected from 280 sporadic and 389 familial NF1 patients. We excluded 11 (3.9%) exceptionally mild sporadic cases as possible somatic mosaics and 14 (5.0%) sporadic cases with the large deletion phenotype. We compared paternal age in the remaining 255 sporadic probands to 100 familial probands for whom both the paternal and maternal ages at birth were known. The mean paternal age for the sporadic probands (31.71 years) was significantly greater ($p = 0.017$) than that for familial probands (29.87 years). The results were unaffected by ethnicity or year of ascertainment.

We also studied the effect of maternal age on transmission of the abnormal *NF1* allele. We found no significant difference between the maternal age at birth of 74 proband children of affected mothers and that of 63 proband children of affected fathers. Logistic regression of affection status in 334 children of affected women and 146 children of affected men in 239 families showed no significant relationship with gender of the affected parent, maternal age at birth of the child, paternal age at birth of the child, or birth order. Probands were excluded from this analysis to reduce ascertainment bias. We conclude that there is suggestive evidence for a paternal age effect on the occurrence of sporadic NF1 but that neither maternal nor paternal age at birth affects the occurrence of familial NF1.

VERTEBRAL SCALLOPING IN NEUROFIBROMATOSIS 1:

A QUANTITATIVE APPROACH

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ABSTRACT

Study Design. This is a retrospective study using existing radiographs to measure and compare vertebral scalloping in scoliosis patients with and without neurofibromatosis 1(NF1).

Objective. To investigate quantitative differences in vertebral scalloping between children with and without NF1 who have scoliosis.

Summary of Background Data. Scoliosis occurs in 10-25% of individuals with NF1. Dystrophic bony features such as vertebral scalloping are often present, but the relationship of vertebral scalloping to spinal curve severity and progression is unknown. Vertebral scalloping is often found in NF1 patients who have scoliosis but is rarely seen in the non-NF1 population.

Methods. Lateral radiographs of the lumbar vertebrae measured for 27 children with scoliosis, 13 of whom have NF1 and 14 of whom do not. The distribution of posterior scalloping ratios in each group and the most extreme ratio in each subject in each group were compared.

Results. Scalloping ratios from the NF1 patients were not normally distributed, with 31% of these patients exhibiting ratios greater than 1.20. Scalloping ratios from the non-NF1 patients were normally distributed, with a mean of 1.13 and a standard deviation of 0.03. The distribution was significantly different between the groups.

Conclusions. In non-NF1 children with scoliosis there was a range of mild scalloping whereas a subset of children with NF1 has severe scalloping. Further studies are needed to determine the possible role of vertebral scalloping in scoliosis severity and progression in patients with NF1.

Key Words: neurofibromatosis 1, vertebral scalloping, scoliosis, vertebral dysplasia

MINI ABSTRACT

This retrospective analysis measured and compared vertebral scalloping from children with scoliosis, 13 with and 14 without NF1. Vertebral scalloping ratios differed between patients with and without NF1. Our findings are consistent with the existence of two subgroups of NF1 patients with scoliosis: those with vertebral scalloping and those without.

INTRODUCTION

Neurofibromatosis 1 (NF1) is an autosomal dominant genetic disease with a prevalence in childhood of about 1 in 3000 [2,6,15]. The responsible gene, *NF1*, has been identified and mapped to the long arm of chromosome 17 [1]. NF1 is extremely variable clinically, but characteristic features include peripheral neurofibromas, café-au-lait macules and Lisch nodules [13].

NF1 produces skeletal abnormalities in a large proportion of affected patients. Scoliosis is the most common skeletal manifestation in NF1, with a prevalence that has been estimated between 10 and 60% in various studies [7,8]. The most common form of scoliosis in NF1 is a single curve involving 4-6 thoracic vertebrae [7], but there is great variation in the type and severity of spinal curvature among NF1 patients. Many NF1 patients with scoliosis have associated features (classically called *dystrophic* features) like vertebral scalloping, rib penciling, paravertebral soft tissue mass, or defective pedicles [3,14]. Scoliosis in NF1 is, therefore, classified into two main types: 1) that with associated dystrophic features, and 2) that without [7,11].

Vertebral scalloping refers to an exaggerated concavity of the dorsal surface of a vertebra [12], although there is currently no accepted definition that clearly distinguishes between vertebral scalloping and the normal slight concavity of healthy vertebral bodies. Scalloping of the vertebrae occurs at an extremely young age in NF1. In Funasaki's study of 65 NF1 patients with scoliosis [7], 31 had scalloping that was apparent by the age of ten. Eleven of these patients were followed for an average of 14 years each, and no one developed new vertebral scalloping after age ten. According to Casselman and Mandell[3], posterior scalloping is a more characteristic finding than anterior or lateral scalloping among NF1 patients.

The cause of vertebral scalloping in NF1 has not been determined and may differ from case to case [14]. Dural ectasia, which is an increase in the thickness of the thecal sac, can cause expansive and erosive deformities to adjacent vertebral bodies [11]. Scalloping may also be caused by erosion from nearby neurofibromas, or even by primary mesodermal dysplasia of meninges. Finally, abnormal development of the vertebral tissues (i.e., true vertebral dysplasia) may also give rise to scalloping. Vertebral scalloping may be seen in patients with dysplastic scoliosis, such as those seen secondary to Marfan's syndrome.

The goal of this study was to characterize vertebral scalloping quantitatively in NF1 and non-NF1 patients with scoliosis, and to identify any differences between the two populations.

METHODS

Sample Population

This study involved reviewing available AP and lateral spinal radiographs from 27 patients with scoliosis followed in the Scoliosis Clinic at British Columbia Children's Hospital. The study included two groups: 13 children with NF1 and scoliosis, and 14 children with idiopathic scoliosis. The children with NF1 at examination ranged from 3-18 years old and had primary curves ranging from 36°–75° (average=52.7°). The non-NF1 group, identified through the clinic database, ranged from 7-17 years old at examination and had primary curves ranging from 30°– 90° (average=51.0°).

Measuring Scalloping

Vertebral scalloping was measured from lateral radiographs of the lumbar spine only. The lateral films were scanned into a PC computer and stored as digital images. Measurements were obtained using a digital measurement tool in Adobe Photoshop 5.5. This method allowed for better visualization and enhanced accuracy and reliability. All measurements were performed blindly by the first author.

Vertebral scalloping was estimated as a ratio using a modification of the procedure described in Funasaki's study [7]. Three width measurements were obtained for each individual vertebral body: superior, inferior, and waist (Figure 1). The average of the superior and inferior measurements was divided by the waist measurement to obtain a "scalloping ratio". If there is no scalloping (no indentation in either the anterior or posterior edge), a ratio of 1.0 will be measured. A ratio greater than 1.0 indicates scalloping: the larger the ratio, the more severe the vertebral scalloping.

Data Analysis

An individual patient may have more than one scalloped vertebral body, but we used only the largest scalloping ratio from each patient for analysis to maintain the statistical independence of each case. For those patients who had more than one set of radiographs at different ages, ratios were obtained from all the sets, and the largest scalloping ratio measured for each patient was used in this analysis. The distribution of maximum scalloping ratios in each group was tested for normality with a Shapiro-Wilks test. The distributions of maximum scalloping ratios in the NF1 and non-NF1 groups were compared using a Mann-Witney U test. Comparisons with $p < 0.05$ were considered to be statistically significant.

RESULTS

Scalloping ratios were calculated from lateral radiographs of the lumbar vertebrae in 27 children with scoliosis -- 13 NF1 patients and 14 non-NF1 patients. The mean for the maximum scalloping ratio for the NF1 patients with scoliosis was 1.18 (±0.13). For the scoliosis patients without NF1, the mean was 1.13 (±0.026). The much greater variability of values in the NF1 group is also seen in Figure 2, which shows the frequency distribution of values for the largest scalloping ratio obtained in each case. The distributions of maximum scalloping ratios for NF1 patients and those without NF1 were clearly different. The distribution of scalloping ratios in scoliosis patients without NF1did not differ significantly from normal ($p=0.62$), but the distribution among NF1 patients does ($p=0.02$). None of the non-NF1 patients in this sample had maximum scalloping ratios above 1.21 (3 SD's above mean of the normal distribution), while 4 out of the 13 NF1 patients did. On the basis of the normal distribution observed for non-NF1

cases, we estimated the probability that a randomly-chosen non-NF1 patient with scoliosis had a lumbar scalloping ratio of greater than 1.21 to be 0.0028. The distributions for the maximum scalloping ratios did not differ significantly ($p=0.61$, Mann-Witney U test) between these small groups.

DISCUSSION

This study showed there was a group of patients with NF1 who had significant vertebral lumbar scalloping. This new evidence is promising for developing further diagnostic criteria in young patients with NF1 who present with only a few of the clinical criteria for NF1. As we observed, the scalloping appeared in young patients as well as older patients. Conclusive diagnostic criteria could be made with a larger population sample across more ethnic groups with a multi-center study.

The method of measuring the images digitally enhanced the visualization accuracy however the images were still two-dimensional. The superior, waist, and inferior measurements varied considerably depending on the three-dimensional orientation of the patient and individual vertebrae when the radiograph was taken. Without three-dimensional imaging available, this problem was difficult to overcome. Three-dimensional CT's may be the best accurate method to assess vertebral scalloping however, it is costly and exposes the patient to increased radiation.

Another limitation of the study was that we only measured the lumbar vertebrae. Salerno and Edeiken [14] suggest that scalloping of vertebral bodies is most frequent on the posterior surface. Posterior vertebral scalloping was best visualized on lateral radiographs as a concave indentation of the posterior margin of a vertebral body towards

the center [3]. We used measurements from lumbar vertebrae for this study because they provided the best quality images of large vertebral bodies that were free from overlap from ribs. However, we know that vertebral scalloping in NF1 patients is often focal[3,12,14], so some patients who do not have vertebral scalloping in the lumbar region may have it elsewhere in the spine. Further study of scalloping in other sections of the spine is needed to determine how representative our findings are for the whole vertebral column.

Funasaki *et al* [7] used a single measurement for each vertebral body to determine the presence orabsence of scalloping: they took the distance from the posterior limit to the deepest anterior point of the posterior margin and diagnosed scalloping if this value was greater than 4mm in a lumbar vertebra. This method did not account for differences that occur in the sizes of vertebral bodies from different patients, in children of different ages, or in different regions of the spine. We used a ratio of vertebral measurements in this study to allow vertebrae of different sizes to be compared directly.

The maximum scalloping ratios in lumbar vertebrae of children with scoliosis who do not have NF1 were normally distributed, but this was not true for children with scoliosis who have NF1 (Figure 2). Vertebral scalloping, defined as a scalloping ratio more than 3 SD's above the mean for the non-NF1 group with scoliosis, was seen in the lumbar vertebrae of 4 of the 13 NF1 patients studied. The distribution of scalloping ratios in other 9 NF1 patients was similar to that in patients without NF1, so it appears that there were two classes of NF1 patients with scoliosis – those with and those without vertebral scalloping. This conclusion is consistent with clinical experience [77,11] but

cannot solely be made on the basis of the current study, in which we just examined vertebral bodies of the lumbar spine.

CONCLUSION

The pathogenesis of vertebral scalloping in NF1 is unknown. A recent study by Durrani *et al* [5] suggests that "dystrophic" features may be correlated with clinical progression of a spinal deformity, but no independent effect was shown for vertebral scalloping. Our current study indicated there may be a subset of NF1 patients who had increased lumbar vertebral scalloping however, long-term longitudinal studies of vertebral scalloping measured quantitatively in NF1 patients are required to determine whether scalloping is progressive. As well as to clarify its relationship, if any, to the severity and progression of scoliosis.

REFERENCES

1. Barker, D *et al.* Gene for von Recklinghausen neurofibromatosis is in the pericentric region of chromosome 17. Science 1987; 236:1100-1102.

2. Brill, CB. Neurofibromatosis: Clinical Overview. Clinical Orthopaedics 1989; 245:10-15.

3. Casselman, ES, Mandell, GA. Vertebral Scalloping in Neurofibromatosis. Radiology 1979; 131:89-94.

4. Clementi, M *et al.* Neurofibromatosis-1: A Maximum Likelihood Estimation of Mutation Rate. Human Genetics 1990; 84:116-118.

5. Durrani, AA, *et al.* Modulation of Spinal Deformities in Patients with NF Type 1. Spine 2000; 25(1):69-75.

6. Friedman, JM. Epidemiology of Neurofibromatosis Type 1. American Journal of Medical Genetics 1999; 89(1):1-6.

7. Funasaki, H. *et al.* Pathophysiology of Spinal Deformities in Neurofibromatosis. Journal of Bone and Joint Surgery 1994; 76(5):692-700.

8. Hunt, JC, and Pugh, DG. Skeletal Lesions in Neurofibromatosis. Radiology 1961; 76:1-20.

9. Huson, SM, Compston, DAS, and Harper PS. Von Recklinghausen Neurofibromatosis: A Clinical and Population Study in South-East Wales. Brain 1988; 111:1355-1381.

10. Kim, FM, Poussaint, TY, and Barnes, PD. Neuroimaging of Scoliosis in Childhood. Pediatric Neuroimaging 1999; 9(1):195-221.

11. Kim, HW, Weinstein, SL. Spine Update: The Management of Scoliosis in Neurofibromatosis. Spine 1997; 22(23):2770-2776.

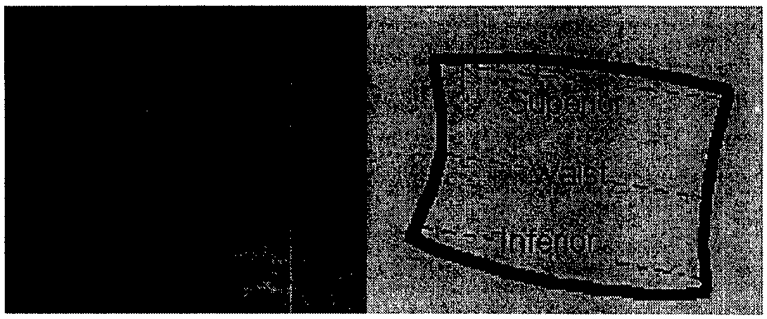
12. Mitchell, GE, Lourie, H, and Berne, AS. The Various Causes of Scalloped Vertebrae with Notes on Their Pathogenesis. Radiology 1967; 89:67-74.

13. Riccardi, VM, and Eichner, JE. 1992. Neurofibromatosis: Phenotype, Natural History, and Pathogenesis. Baltimore, MD, Johns Hopkins University Press.

14. Salerno, NR, Edeiken, J. Vertebral Scalloping in Neurofibromatosis. Radiology 1970; 97:509-510.

15. Viskochil, D. Neurofibromatosis 1 – Introduction. American Journal of Medical Genetics 1999; 89(1):v-viii.

LIST OF FIGURES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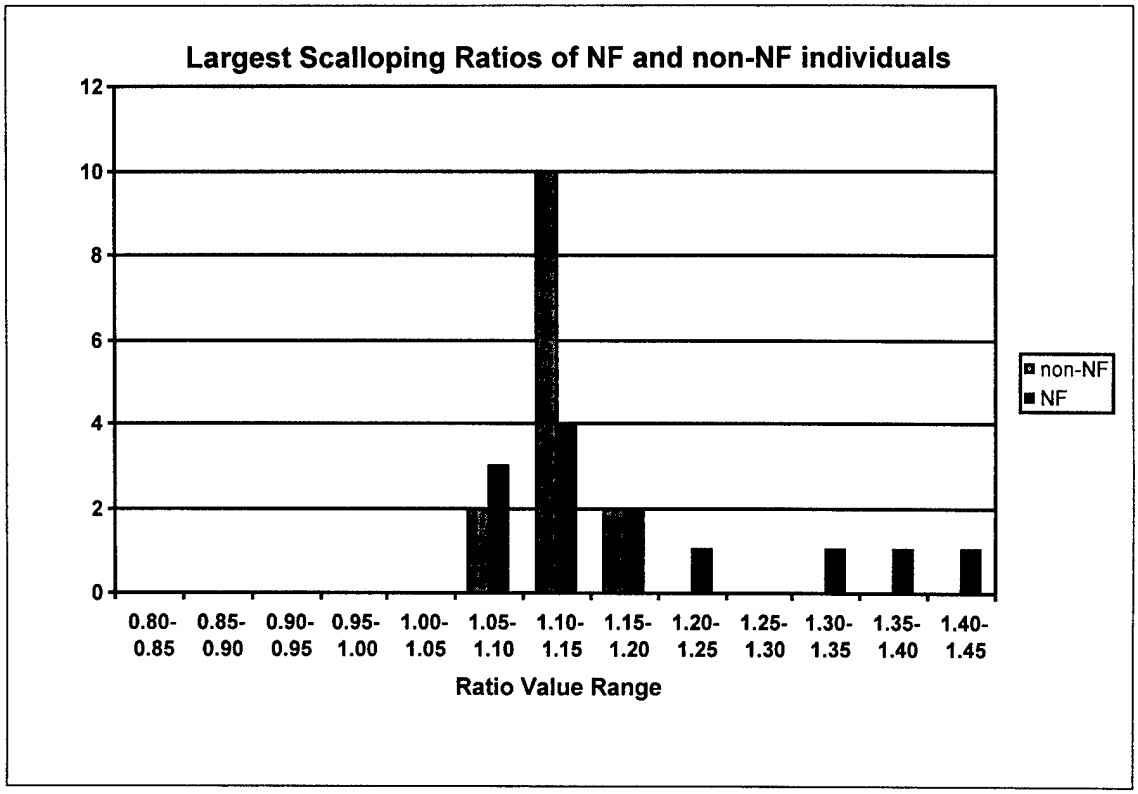
Largest Scalloping Ratios of NF and non-NF individuals
12
10
8
6
4
2
0
0.80-0.85
0.85-0.90
0.90-0.95
0.95-1.00
1.00-1.05
1.05-1.10
1.10-1.15
1.15-1.20
1.20-1.25
1.25-1.30
1.30-1.35
1.35-1.40
1.40-1.45
Ratio Value Range
non-NF
NF

Vertebral scalloping in Neurofibromatosis (NF1): A quantitative approach. *E. Kwok[1], B. Sawatzky[2], P. Birch[1], J.M. Friedman[1], S. Tredwell[2].* 1) Department of Medical Genetics, University of British Columbia, Vancouver, BC, Canada; 2) Department of Orthopaedics, BC Children's Hospital, Vancouver, BC, Canada.

Scoliosis occurs in 10-25% of individuals with NF1. Vertebral scalloping is often found in NF1 patients, but its clinical significance remains unknown. We measured and compared vertebral scalloping in children ascertained from a pediatric tertiary referral hospital. We used lateral radiographic films of lumbar vertebrae from 13 NF1 children with scoliosis and 14 non-NF1 scoliosis patients to derive a scalloping ratio, which was estimated by taking three width measurements from each vertebra: superior, inferior, and waist. Digital images of the radiographs were measured using electronic calipers. The average of the superior and inferior measurements was divided by the waist measurement to obtain a scalloping ratio for each lumbar vertebra.

Only the highest ratio from each patient was used in data analysis. Scalloping ratios from the non-NF1 patients were normally distributed, with a mean of 1.13 and a standard deviation of 0.03. A Z-test gave a probability of 0.003 that a randomly chosen non-NF1 patient will have a ratio greater than 1.20. Scalloping ratios from the NF1 patients are bimodal, with 31% exhibiting ratios greater than 1.20.

We conclude that this method provides a useful and cost effective way to characterize vertebral scalloping in children with NF1. There appear to be two groups of NF1 patients with scoliosis: those with vertebral scalloping and those without. Further studies are needed to define the natural history and pathogenesis of vertebral scalloping and to determine whether it contributes to the severity or progression of scoliosis in NF1 patients.

This study was funded in part by the British Columbia Neurofibromatosis Foundation. Details can be found at: http://www.medgen.ubc.ca/friedmanlab/index.html.

UNIDENTIFIED BRIGHT OBJECTS ASSOCIATED WITH FEATURES OF NEUROFIBROMATOSIS 1

Running Title: UBOs in NF1

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ABSTRACT

Unidentified bright objects are commonly observed on MRI in young neurofibromatosis 1 patients, but their clinical and pathological significance is largely unknown. Diagnostic features of NF1 include café-au-lait spots, intertriginous freckling, Lisch nodules, neurofibromas, bony lesions and optic glioma. We investigated the relationship between Unidentified bright objects and other features of neurofibromatosis 1. 523 neurofibromatosis 1 patients between the ages of 2 and 20 years who had cranial MRI examinations were selected from the National Neurofibromatosis Foundation International Database. The presence or absence of unidentified bright objects, diagnostic features of neurofibromatosis 1, and central nervous system neoplasms was known in these patients. Logistic regressive models were used to measure associations between unidentified bright objects and the other features while controlling for age.

The occurrence of unidentified bright objects was found to be associated with the number of diagnostic features, but most significantly with Lisch nodules (Odds Ratio=1.6, 95% Confidence Interval=1.1-2.3), subcutaneous neurofibromas (OR=2.0, 95%CI=1.3-3.1), optic gliomas (OR=2.1, 95%CI=1.2-3.6), and other CNS neoplasms (OR=9.0, 95%CI=1.2-70). These findings suggest a common causal mechanism between unidentified bright objects and these cardinal clinical features in children with neurofibromatosis 1

INTRODUCTION

"Unidentified bright objects" (UBOs) have been observed as areas of increased T2-weighted signal intensity on MRI in 43-93% of children with neurofibromatosis 1 (NF1) [1]. They are almost never seen in NF1 patients over the age of 20 years [2]. Pathologically, UBOs may represent increased fluid within the myelin associated with hyperplastic or dysplastic glial proliferation [3] and may be associated with cognitive deficits [4]. We show here that UBOs may also be associated with the occurrence of other clinical features in young (<21 years) NF1 patients.

METHODS

Patients

523 patients between the ages of 2 and 20 years who met the NIH Diagnostic Criteria for NF1 and had cranial MRI examinations were selected from the National NF Foundation International Database (NFDB) [5]. The presence or absence of UBOs on cranial MRI examination was determined for each patient [6]. The presence or absence of CNS neoplasms (other than optic glioma) and of the following NIH Diagnostic features [7] was known in each patient: ≥6 café-au-lait spots of sufficient size for age, intertriginous freckling, ≥2 Lisch nodules, ≥2 neurofibromas or one plexiform neurofibroma, a typical bony lesion and optic glioma. Although an affected first-degree relative is also one of the NIH Diagnostic Criteria and accounts for the fact that some of the patients included had only one diagnostic feature of NF1, family history was not considered in this analysis.

Statistical Analysis

The χ^2 and Cochran-Armitage trend statistics were used to test whether the likelihood of UBOs was independent of the number of diagnostic features [8]. Logistic regression was used to quantify the associations between UBOs and each of the diagnostic features simultaneously, while controlling for age and gender [9]. Features with $p>0.10$ in the first logistic regressive model were excluded. Parameters for the remaining features were re-estimated and second order interactions were evaluated.

RESULTS

Frequency of clinical features analysed

259 (50%) of 523 NF1 patients in the NFDB had UBOs, 492 (94%) had ≥6 café-au-lait spots, 426 (81%) intertriginous or axillary freckling, 253 (48%) Lisch nodules, 128 (24%) ≥2 subcutaneous neurofibromas, 85 (16%) ≥2 cutaneous neurofibromas, 100 (19%) ≥1 plexiform neurofibromas, 73 (14%) a typical bony lesion, 70 (13%) had optic glioma - 23 (4%) symptomatic and 47 (9%) asymptomatic. 11 (2%) patients had other CNS neoplasms - 7 non-optic gliomas, 1 ependymoma, 1 ganglioneuroma, 1 hamartoma of the pons and midbrain, and 1 hypothalamic neoplasm.

UBOs associated with diagnostic features

Figure 1 shows the frequency by age of UBOs among 523 NF1 patients 2-20 years of age. Figure 2 shows the frequency of UBOs according to the number of diagnostic features present in each patient. The frequency of UBOs in our sample does not increase with age in this range (Figure 1), but increases from 29% in patients who

have only one diagnostic feature to 100% in patients who have six features (Figure 2) (χ^2=28.0, p<.0001; Cochran-Armitage Trend = –5.201, p<.001). The mean age of patients with 1 or 2 diagnostic features was 8 years and the mean age of those with 4, 5 or 6 diagnostic features was 12 years.

Table 1 summarizes the associations between UBOs and other clinical features estimated by multi-covariate logistic regression. Model 1 shows that café-au-lait spots, freckling, cutaneous and plexiform neurofibromas and characteristic bony lesions are no more likely to be present in NF1 patients with UBOs than in NF1 patients without UBOs. These variables were excluded and the remaining associations recalculated in Model 2. Model 2 shows that Lisch nodules, subcutaneous neurofibromas, optic gliomas, and other neoplasms are more likely to be present in NF1 patients with UBOs than in NF1 patients without UBOs. Age, gender and all second order interactions were not significant.

DISCUSSION

We found UBOs to be associated with the number of diagnostic features in young NF1 patients (Figure 2), but most significantly with optic gliomas, other CNS neoplasms, subcutaneous neurofibromas, and Lisch nodules (Table 1). The percentage of patients with Lisch nodules and neurofibromas increases with age [10]. However, our approach adjusts for age (and gender), so the observed associations are not confounded by age and are not an artifact of age-specific sampling. The frequencies of these features in our patients are similar to those reported for other patients in this age group [1,11].

The patients in this study were selected from the NFDB because they had cranial MRI. Most of the centres that contribute data to the NFDB do not routinely do MRI

examinations on all young NF1 patients, in accordance with current recommendations [7]. Therefore, we were concerned that the patients who did have MRIs were more likely to have symptoms of intracranial pathology. The frequency of such intracranial lesions as optic and other CNS gliomas was not unusually high compared to prospective and population based studies [1,11]. Nevertheless, an inclusion bias has to be considered. MRIs on our patients were interpreted by a different radiologist at each centre. Most of our patients were seen at pediatric clinics[5] and agreement between different pediatric radiologists with respect to the presence or absence of UBOs on MRIs of NF1 patients has been shown to be around 85% [6]. Therefore, we expect the criteria for defining UBOs and differentiating them from initial low-grade gliomas or other brain lesions to be less consistent than if all patients had been examined by the same radiologist.

Patient information was not kept from the radiologists who interpreted the MRI scans and they may have been more likely to recognise UBOs if a diagnosis of NF1 had already been established or was obvious on clinical or radiological examination. This is unlikely to explain why NF1 patients with UBOs were more likely to also have such non-promonient features as Lisch nodules than NF1 patients without UBOs. Moreover, NF1 patients with UBOs were no more likely to also have two of the most obvious diagnostic features (café-au-lait spots and cutaneous neurofibromas) than NF1 patients without UBOs.

A longitudinal MRI study by Griffiths et al. found brain tumours in several children with NF1 that developed at the site of a previously-recognised UBOs [1]. In addition, the children who developed brain tumours tended to have more UBOs than other children their age with NF1. We did not analyse the precise location or number of

UBOs in our patients, and our data are cross-sectional, not longitudinal. Also, follow-up may help define some of the lesions labeled as UBOs. Nevertheless, we did observe that NF1 patients with UBOs were more likely to also have CNS gliomas than NF1 patients without UBOs.

The cross-sectional nature of our data preclude conclusions about whether UBOs develop before or after the other associated features in individual NF1 patients. It would be important to assess this prospectively because UBO are more prevalent at a younger age than Lisch nodules, subcutaneous neurofibromas and neoplasms [6] and might predict the future development of more serious problems in young NF1 patients. For this reason they have been proposed as an additional diagnostic criterion in children [12]. UBOs may turn out to be a reliable diagnostic criterion, but not before they are better defined in terms of location, size and number and their sensitivity and specificity are properly studied [6].

Our observations support a pathogenic relationship between UBOs and diagnostic features in young NF1 patients. The common thread between the associated features (optic gliomas, other neoplasms, Lisch nodules, and subcutaneous neurofibromas) may be dysregulated cellular proliferation resulting from haploinsuffiency of neurofibromin [13], but it is hard to understand what this has to do with UBOs if they are simply areas in which the myelin is immature and contains increased fluid. The key may be underlying hyperplastic or dysplastic glial cell proliferation [3], which leads to formation of the altered myelin in UBOs. If widespread dysregulation of glial cell proliferation in the brains of young NF1 patients is involved in the pathogenesis of UBOs, the mechanism could be similar to that underlying the formation of optic and other CNS gliomas [14],

and, by analogy, to the formation of Lisch nodules and subcutaneous neurofibromas in these patients. In other words, UBO's may be markers for the severity of cellular dysfunction that also predisposes children to these other associated features of NF1.

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REFERENCES

[1] **Griffiths** PD, Blaser S, Mukonoweshuro W, Armstrong D, Milo-Mason G, Cheung S. Neurofibromatosis bright objects in children with neurofibromatosis type 1: a proliferative potential? Pediatrics 1999;104:e49. (pp1-8)

[2] **DiMario** F, Ramsby G. Magnetic resonance imaging lesion analysis in neurofibromatosis type 1. Arch Neurol 1998;55:500-505.

[3] **DiPaolo** DP, Zimmerman RA, Rorke LB, Zackai EH, Bilaniuk LT, Yachnis AT. Neurofibromatosis type 1: pathologic substrate of high-signal-intensity foci in the brain. Radiology 1995;195:721-4.

[4] **North** K, Riccardi V, Samango-Sprouse C, Ferner R, Moore B, Legius E, Ratner N, Denckla M. Cognitive function and academic performance in neurofibromatosis 1: Consensus statement from the NF1 Cognitive Disorders Task Force. Neurology 1997;48:1121-1127.

[5] **Friedman** J, Greene C, Birch P, and the NNFF International Database P. National Neurofibromatosis Foundation International Database. Am J Med Genet 1993;45:88-91.

[6] **DeBella** K, Poskitt K, Szudek J, Friedman JM. Use of unidentified bright objects on MRI for diagnosis of neurofibromatosis 1 in children. Neurology 2000;54:1646-51.

[7] **Gutmann** D, Aylsworth A, Carey J, Korf B, Marks J, Pyeritz R, Rubenstein A, Viskochil D. The diagnostic evaluation and multidisciplinary management of neurofibromatosis 1 and neurofibromatosis 2. JAMA 1997;278:51-57.

[8] **Zar** JH. Biostatistical analysis. Chapter 24, "More on Dichotomous Variables," pp565-8. Upper Saddle River, N.J.: Prentice Hall, 1999.

[9] **SAS**. Statistical analysis software. 6.12 ed. SAS Institute, Cary, NC, 1996.

[10] **DeBella** K, Szudek J, Friedman JM. Use of the national institutes of health criteria for diagnosis of neurofibromatosis 1 in children. Pediatrics 2000;105:608-14.

[11] **McGaughran** JM, Harris DI, Donnai D, Teare D, MacLeod R, Westerbeek R, Kingston H, Super M, Harris R, Evans DG. A clinical study of type 1 neurofibromatosis in north west England. J Med Genet 1999;36:197-203.

[12] **Curless** R, Siatkowski M, Glaser J, Shatz N. MRI diagnosis of NF-1 in children without café-au-lait skin lesions. Pediatr Neurol 1998;18:269-271.

[13] **Gutmann** DH, Loehr A, Zhang Y, Kim J, Henkemeyer M, Cashen A. Haploinsufficiency for the neurofibromatosis 1 (NF1) tumor suppressor results in increased astrocyte proliferation. Oncogene 1999;18:4450-9.

[14] **Friedman** J, Birch P. An association between optic glioma and other tumours of the central nervous system in neurofibromatosis type 1. Neuropediatrics 1997;28:131-132.

Table 1: Features associated with Unidentified Bright Objects (UBOs) in 523 NF1 Patients. Logistic regression was used to quantify the associations between UBOs and each of the diagnostic features simultaneously, while controlling for age and gender. Age, gender and features with $p<0.10$ in Model 1 were included in Model 2. There were no significant second order interactions between the explanatory features in Model 2.

	Model 1	**Model 2**		
Main Effects	*P-Value*	*P-Value*	*Odds Ratio*	*95% Confidence Interval*
Age	0.83	0.75	0.99	0.95-1.03
Male Gender	0.10	0.10	0.74	0.52-1.05
Café-au-Lait Spots	0.18	—	—	—
Freckling	0.32	—	—	—
Lisch Nodules	0.04	0.02	1.59	1.10-2.32
Neurofibromas				
Cutaneous	0.95	—	—	—
Subcutaneous	0.004	0.002	2.00	1.30-3.08
Plexiform	0.18	—	—	—
Bony Lesion	0.59	—	—	—
Optic Glioma	0.01	0.009	2.08	1.20-3.62
Other CNS Neoplasms	0.04	0.04	8.99	1.13-69.97

Figure 1: Prevalence by age of Unidentified Bright Objects (UBOs). The curve is based on 523 neurofibromatosis 1 (NF1) patients from the National Neurofibromatosis Foundation International Database who had cranial MRI. Dotted lines indicate 95% confidence intervals.

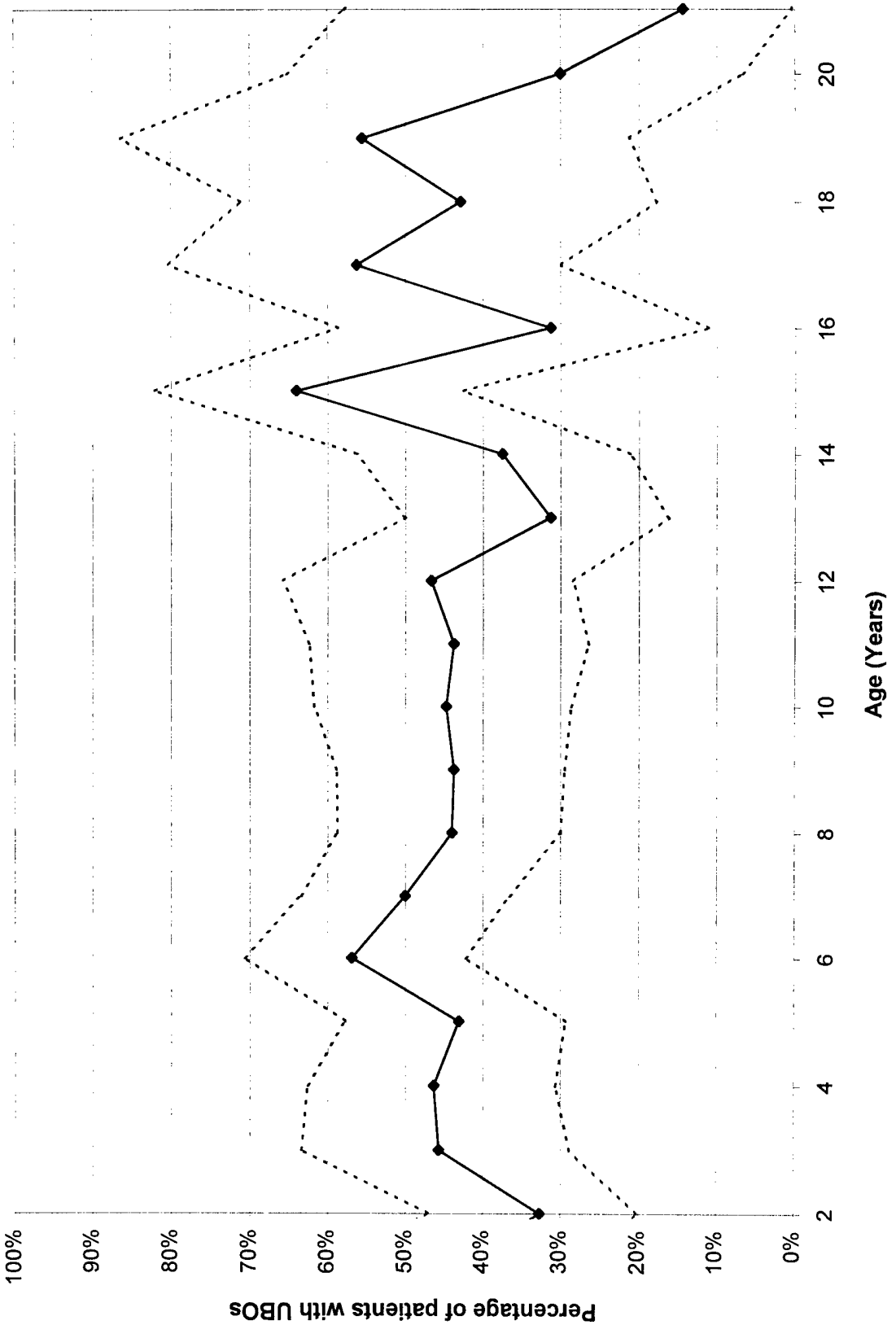
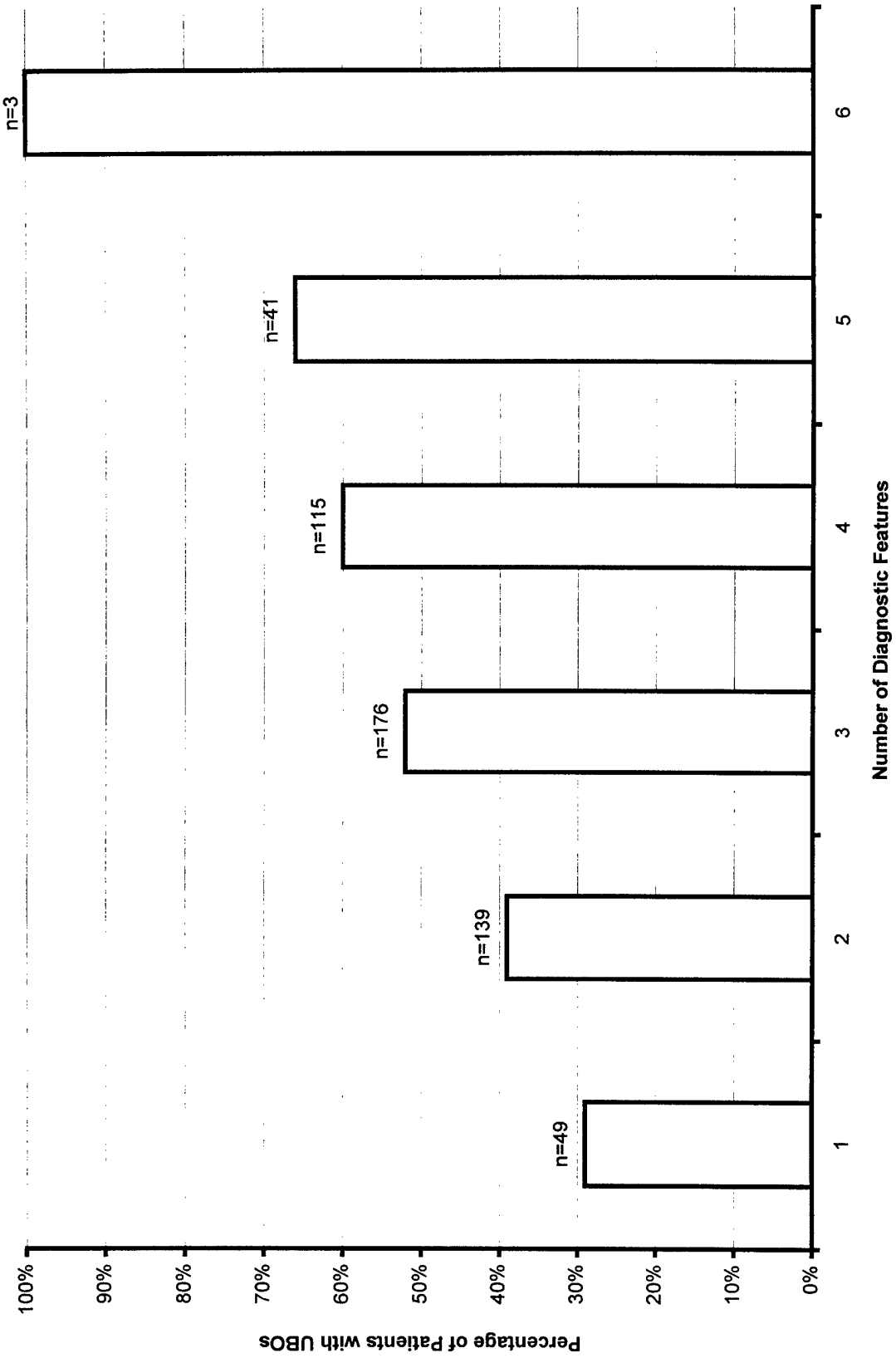

Percentage of patients with UBOs
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
2
4
6
8
10
12
14
16
18
20
Age (Years)

Figure 2: Unidentified Bright Objects versus the number of diagnostic features. Percentage of Unidentified Bright Objects on MRI by the number of diagnostic features in 523 NF1 patients between 2 and 20 years of age from the National Neurofibromatosis Foundation International Database. The label above each column indicates the number of patients with the respective number of diagnostic features.


Percentage of Patients with UBOs
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
n=49
n=139
n=176
n=115
n=41
n=3
1
2
3
4
5
6
Number of Diagnostic Features

LOGISTIC REGRESSIVE MODELS OF CLINICAL FEATURES IN NEUROFIBROMATOSIS 1 (NF1)

Running Title: Logistic Regression in NF1

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SUMMARY

Neurofibromatosis 1 (NF1) is a common, fully penetrant, autosomal dominant disease. The clinical course is generally progressive but highly variable, and the pathogenesis is poorly understood. A better understanding of this variability may shed light on its pathogenesis.

We studied interactions among 13 of the most common or important NF1 clinical features in data on 2797 NF1 probands (divided into separate developmental and validation subsets) and 511 of their affected relatives from the NNFF International Database and on 441 NF1 patients from a population-based registry in north-west England. We developed logistic regressive models for each of the 13 features using the developmental sample and attempted to validate these models in the other 3 samples. Age and gender were included as covariates in all models.

Models were successfully developed and validated for 10 of the 13 features analyzed. The results are consistent with grouping 9 of the features into three sets of associated features: 1) café-au-lait spots, intertriginous freckling and Lisch nodules; 2) cutaneous, subcutaneous and plexiform neurofibromas; and 3) macrocephaly, optic glioma and other neoplasms. In addition, 3-way interactions among café-au-lait spots, intertriginous freckling and subcutaneous neurofibromas suggest that the first two groups are not independent.

Clinical features within a group may share pathogenic mechanisms that differ, at least in part, from those underlying features in other groups. The results suggest a variety of familial and molecular investigations into the pathogenesis of NF1.

KEY WORDS

phenotype, expressivity, database, pathogenesis

INTRODUCTION

Neurofibromatosis 1 (NF1) expressivity is tremendously variable [Friedman et al. 1999], but subtle phenotypic patterns may exist within subgroups of affected patients. The existence of such subgroups is supported by the observation of a relatively consistent phenotype among patients with deletions of the entire *NF1* gene and in families with NF1 variants such as Watson syndrome [Upadhyaya et al. 1990; Allanson et al. 1991]. In these cases, particular genotypes result in specific constellations of clinical features.

In a previous study, we demonstrated several associations between pair-wise combinations of clinical features among age-stratified probands with NF1 [Szudek et al. in press]. These analyses support the existence of phenotypic subgroups but were limited in two ways: only two features could be examined at once and some of the comparisons may have been confounded by age. Although we analyzed age in 10-year strata, there still may have been considerable age-related variability, especially among the youngest patients. In this study, we have extended our analysis of associations among clinical features in NF1 patients by using logistic regression to consider joint and interactive effects of several clinical features at once and to control for age as a continuous variable. Our findings clarify and refine the associations among clinical features in NF1 patients and provide further clues to the pathogenesis of these features.

SUBJECTS AND METHODS

This study involved analysis of four separate clinical samples of patients with NF1 – the developmental, validation, relative, and population-based Manchester samples, as described below. Logistic regressive models were built from an initial series of univariate models, by progressively adding covariates and interaction terms, in the developmental sample. The best fitting of these models were then tested in each of the other samples, using both the parameters from the developmental sample and by refitting the parameters in each of the other samples.

Subjects

Subjects were obtained from two large clinical databases: the National Neurofibromatosis Foundation International Database (NFDB) and the Manchester NF1 database (MANF1). All patients included in this analysis were diagnosed with NF1 according to established clinical criteria [NIH 1988; Gutmann et al. 1997]. The NFDB includes extensive demographic and cross-sectional clinical information on 2797 NF1 probands and 511 of their affected relatives examined since 1980 at 25 participating centres in North America, Europe and Australia. 83% of the cases are Caucasian, 7% Asian, 4% African-American, 6% other or mixed race. Subject age at exam ranged from birth to 89 years. All information was collected and recorded on each patient using a standard procedure [Friedman and Birch 1997]. The data were audited for quality and consistency by the NFDB administrator. The Manchester NF1 Database (MANF1) is a population-based registry of north-west England and includes clinical information on 270

probands, 94 affected parents and 140 of their affected children [McGaughran et al. 1999]. Probands and affected relatives were studied as a single group in the MANF1 because there were not enough cases to permit separate analysis. 92% of the cases are Caucasian, 4% Indian, 2% Black, 1% Bangladeshi and 1% Pakistani. There is no overlap among the patients included in the NFDB and MANF1 databases.

Clinical Features

We selected 13 important or frequent clinical features of NF1 for this study: café-au-lait spots, intertriginous freckling, discrete cutaneous neurofibromas, discrete subcutaneous neurofibromas, diffuse or nodular plexiform neurofibromas (referred to as "plexiform neurofibromas"), Lisch nodules, scoliosis, tibial or other long bone bowing or pseudarthrosis ("pseudarthrosis"), optic glioma, macrocephaly, short stature, seizures and neoplasms (other than neurofibromas or optic glioma). Each of these features was coded as either "present", "absent" or "unknown". Age, coded to the nearest 0.01 year, and gender were considered as covariates.

Most of the features were identified by physical examination. Discrete neurofibromas were coded as "present" if the subject had two or more cutaneous or subcutaneous neurofibromas. Short stature was coded as "present" if the subject's height was 2 or more standard deviations below the age- and sex-matched population mean. Subjects with pseudarthrosis, early or delayed puberty, scoliosis, vertebral dysplasia, or spinal compression were excluded from analyses involving height because these features may alter what the height would otherwise be. Macrocephaly was coded as "present" if the subject's head circumference was 2 or more standard deviations above the age- and

sex- matched population mean. Subjects with plexiform neurofibroma of the head, early or delayed puberty, or hydrocephalus were excluded from analyses involving head circumference because these features may alter the head circumference. Standard population norms for height and head circumference by age were obtained from the National Center for Health Statistics and the Fels Institute [Hamill et al. 1977]. Lisch nodules were diagnosed or excluded by a slit lamp examination; individuals who did not have a slit lamp examination were coded as "unknown". The presence or absence of optic glioma was determined by cranial MRI or CT examination; individuals who did not have cranial imaging were coded as "unknown". Patients coded as "unknown" for a particular feature were not considered in models involving that feature.

Statistical Models

Thirteen separate logistic regression models were built, with the logit of each of the 13 NF1 features analysed set as the response variable (Y) in a different model. The frequencies of many features change with age, but this effect is not uniform among the features [Friedman et al. 1999]. Therefore, age was controlled as precisely as possible, as a continuous explanatory variable. First, a univariate model was constructed using age as the only covariate:

$$\log\left(\frac{p(1|x)}{1-p(1|x)}\right) = \alpha + \beta_1 AGE$$

where $p(1|x)$ is $Pr(Y=1|$ covariates $x)$.

Maximum likelihood techniques were used to generate parameter estimates [SAS Institute 1996]. Linearity in the logit was examined in each model, and age was transformed when necessary to meet the requirement of linearity in the logit.

$$\log\left(\frac{p(1|x)}{1-p(1|x)}\right) = \alpha + \beta_1 AGETRF$$

where,

$$AGETRF = \exp(-c \times AGE)$$

At *AGE* zero, the value of this function is $\alpha + \beta_1$. For negative values of β_1, the value of *AGETRF* approaches α as *AGE* gets larger. This function approximates the frequency-by-age curves of the NF1 features considered in this study [DeBella et al. 2000]. It was necessary to use this transformation of AGE to maintain linearity of the logit for most outcome variables in this study.

A series of bivariate analyses was then performed using the equation,

$$\log\left(\frac{p(1|x)}{1-p(1|x)}\right) = \alpha + \beta_1 AGETRF + \beta_2 x$$

in which each of the 13 features was set in turn as the response variable (Y) and

AGETRF, and one of the 12 remaining features (x) were used as explanatory variables to screen for potential main effects. Variables with parameters (β's) with $p<0.2$ were included as explanatory variables (x_i's) in multivariate analyses. AGETRF and gender were included as covariates in all models.

$$\log\left(\frac{p(1|x)}{1-p(1|x)}\right) = \alpha + \beta_1 AGETRF + \beta_2 MALE + \beta_3 x_3 + \beta_4 x_4 + \beta_5 x_5 \ldots$$

Following maximum likelihood estimation of the parameters in the multivariate model, the importance of each explanatory variable was reassessed. Explanatory variables with parameters greater than zero with $p<0.2$ were used to refit the model and interaction terms (δ's) among the explanatory variables were considered by forward selection. For example,

$$\log\left(\frac{p(1|x)}{1-p(1|x)}\right) = \alpha + \beta_1 AGETRF + \beta_2 MALE + \beta_3 x_3 + \beta_4 x_4 + \delta_1 x_3 x_4$$

Model Validation

Fitted logistic regressive models always perform favourably on the sample used to generate them [Hosmer and Lemeshow 1989]. Therefore, a random subsample consisting of 1,384 of the 2,797 NF1 probands from the NFDB was excluded, and models

were developed on data from the remaining 1,413 NFDB probands (the "developmental sample"). These models were tested on data from the 1,384 NFDB probands who were originally excluded, the "validation sample". The models were also tested on data from 511 affected relatives of the 2797 NFDB probands and on population-based data from the MANF1, which includes both probands and affected family members. The Hosmer and Lemeshow [1989] goodness-of-fit test was used to asses how well the parameter estimates from the developmental sample fit the validation, affected relative, and MANF1 samples. In addition, parameters for covariates and significant explanatory variables from the best-fitting models derived in the developmental sample were re-estimated by maximum likelihood in the validation, affected relative, and MANF1 samples, to allow more detailed comparison.

Interpretation

Logistic regressive models have a straightforward interpretation in terms of odds-ratios. The strength of interaction between the response variable (Y) and an explanatory variable (x_1) in a univariate model is measured by β_1. Subjects with variable x_1 coded as "present" are $\exp(\beta_1)$ times more likely to also have feature Y than are subjects with feature x_1 absent. The strength of interaction between Y and explanatory variables (x_1 and x_2) in a bivariate model is measured by β_1, β_2, and δ_1. Subjects with variables x_1 and x_2 present are $\exp(\beta_1+\beta_2+\delta_1 x_1x_2)$ times more likely to also have the response feature. Odds ratios with 95% confidence intervals that excluded 1.0 were considered unlikely to

be due to chance alone.

RESULTS

A multivariate logistic regressive model was generated for each of 13 different NF1 clinical features, using age, and gender as covariates and each of the 12 other features as possible explanatory variables. Maximum likelihood parameter estimates were used to determine the best fitting model for each of the clinical features in a developmental sample of NF1 probands from the NFDB, and goodness of fit of each model was then evaluated in three other independent samples – a second, "validation" sample of probands from the NFDB, non-proband affected relatives from the NFDB, and the population-based MANF1 sample that includes both probands and non-probands.

The best-fitting models in the developmental sample for the following outcome features also had an adequate fit in the validation, affected relative and MANF1 samples: intertriginous freckling, subcutaneous neurofibromas, plexiform neurofibromas, optic glioma, pseudarthrosis, macrocephaly, and other neoplasms (Table I). Models for café-au-lait spots, cutaneous neurofibromas, Lisch nodules, seizures, scoliosis and short stature had an inadequate fit in at least one of the samples.

Parameter estimates were independently generated in each of the four samples for the following features: café-au-lait spots, intertriginous freckling, cutaneous neurofibromas, subcutaneous neurofibromas, plexiform neurofibromas, Lisch nodules, scoliosis and short stature (Table II). Parameter estimates for optic glioma, seizures, pseudarthrosis, macrocephaly and other neoplasms could not be generated in all four samples, due to sparseness of data in at least one of the samples. The corresponding cells in Table II are blank.

Some of the parameters from models that had an adequate fit in all four samples were not consistent when generated independently in each sample. In the plexiform model, the parameter estimates for scoliosis and other neoplasms differed greatly among the four samples. In the pseudarthrosis model, the estimate for freckling was inconsistent. Models from the ill-fit samples differed dramatically often by the estimate of only one parameter. The 10 models that had an adequate fit in at least three of the four samples were recalculated including only variables with consistent parameters. Recalculated parameters for the developmental sample are nearly identical to the initial parameters in Table I and are summarized as odds ratios with 95% confidence intervals in Table III. For example, intertriginous freckling was found to be 20% more common in subjects with café-au-lait spots, 40% less common in those with subcutaneous neurofibromas, and 30% more common in those with Lisch nodules. Although only Lisch nodules were significantly associated on their own, freckling was found to be 3.7 times more common in subjects with all three features.

DISCUSSION

The models we have developed include several associations confirmed in two independent samples of NFDB probands, in their affected relatives and in NF1 patients from the population-based MANF1 sample. The NFDB is comprised of patients seen at specialized clinics, so the development and validation samples of probands are probably more severely affected than the NF1 population in general. The affected relative sample was drawn from the same specialized clinics, but their severity is not as biased as that of the probands [Friedman and Birch 1997]. Nevertheless, since half of NF1 cases represent new mutations, and the NFDB only contains data on 511 affected relatives of 2979 probands, it is likely that many affected relatives of these probands are not included in the NFDB. We expect that affected relatives who are included in the NFDB may be more severely affected than those who were not. In contrast, the MANF1 was collected through genetic registries in North-West England by a limited number of physicians. Its ascertainment is near 70% and is thought to be representative of the regional NF1 population [McGaughran et al. 1999]. Model parameters that have been confirmed in all four samples are unlikely to reflect database or specialized clinic biases. Instead these models probably reflect trends that exist in the NF1 population at large.

Features such as optic glioma, seizures, pseudarthrosis naturally fall into a binary coding scheme, while it might be more informative to treat café-au-lait spots, cutaneous and subcutaneous neurofibromas, scoliosis, macrocephaly, short stature and others as ordinal or continuous variables. Although the NFDB contains ordinal data on many variables, the MANF1 contains mostly binary data. All 13 of the features in this study

were treated as binary variables, to avoid uncertainties in the collection of quantitative data from many different NFDB contributing centres and to permit comparison of NFDB models in the MANF1.

Many of the associations in Table III do not have 95% confidence intervals that exclude 1.0. However, several of these models include three-way interactions (Table II), and the first order parameters must be included to adhere to the principle of a hierarchically well formulated model [Kleinbaum 1992]. Also, a variable can contribute to model fit without being significant itself at $p<0.05$, so the criterion for inclusion in a logistic regressive model is often extended to $p<0.2$.

Associations limited to freckling, Lisch nodules, and plexiform, cutaneous and subcutaneous neurofibromas, have been previously reported as pair-wise associations of weak magnitude [Szudek et al. in press]. For example, freckling and Lisch nodules were shown to have a pair-wise age-stratified odds ratio of 1.8 (95% C.I.=1.3-2.4). This study shows that most of these associations not only persist when controlling for other common NF1 features, but increase slightly in strength when the presence of multiple features is considered. The presence of café-au-lait spots, subcutaneous neurofibromas as well as Lisch nodules, make freckling 3.7 (95% C.I.=1.8-7.4) times more likely. Furthermore, this study shows that these pair-wise associations exist side-by-side. For example, cutaneous and subcutaneous neurofibromas are both significantly associated with plexiform neurofibromas (Table III).

The pair-wise association between optic glioma and neoplasms has also been previously reported with an odds ratio of 5.8 [Friedman and Birch 1997a] but gains even more strength when other features are taken into consideration. Optic glioma is 22.4

(95% C.I.= 5.8-86.6) times more common when plexiforms and macrocephaly, as well as neoplasms, are present.

Easton et al. [1993] found evidence of intra-familial correlations in the number of café-au-lait macules and neurofibromas and in the presence or absence of optic gliomas, scoliosis, seizures and referral for remedial education. Easton et al. observed no correlations for head circumference or plexiform neurofibromas. Our study was of individual NF1 patients, not of familial associations, but both studies are consistent with genetic factors contributing to the development of several common NF1 features.

Many of the associations we observed were non-reciprocal – only one of a pair of features appears in the others' model. This suggests that the two features were not of primary importance in accounting for each other's status. This might occur, for example, if both features result from a common pathogenic factor that was not itself included in the models. The reciprocal associations we observed are consistent with the existence of three groups of features among the 13 features studied (figure 1). In general, features were considered to be grouped if each feature appeared as an explanatory variable with a positive parameter estimate in each of the other group members' models. Fundamental pathogenic differences may exist between subjects who have one or more of a group's features and those who do not and the mechanisms shared by associated features may be different for each group of features. However, these NF1 features are not mutually exclusive, and many patients belong to more than one group.

Café-au-lait spots, intertriginous freckles and Lisch nodules are all derived from cells of melanocytic origin [Weston et al. 1981; Perry and Font 1982]. Café-au-lait spots contain melanosomes with giant pigment particles. Intertriginous freckles derive from a

genetic pathway that has nothing to do with light exposure, but they too involve pigment and darken with sun exposure [Fitzpatrick 1981]. Histologically, Lisch nodules are melanocytic hamartomas. Associations between Lisch nodules and pigmentary features have been previously reported [Pietruschka 1961; Zehavi et al. 1986], but the responsible mechanism is unknown.

The associations observed between the occurrence of plexiform, cutaneous and subcutaneous neurofibromas are consistent with the histopathological similarity between these lesions [Harkin and Reed 1969; Burger and Scheithauer 1994]. In addition, each type of neurofibroma is associated with acquired loss or mutation of the normal *NF1* allele in at least some cases [Serra et al. 1997; Sawada et al. 1996]. The negative 3-way interaction terms in two of the three models suggest that associations involving neurofibromas are not independent.

The association between subcutaneous neurofibromas and café-au-lait spots is negative in the café-au-lait spot model, but positive in the subcutaneous neurofibroma model (Table I). This is because the coefficient for subcutaneous neurofibromas in the café-au-lait spot model, changed from positive to negative after adding the interaction term. Similarly, the coefficient for subcutaneous neurofibromas in the intertriginous freckling model, changed from positive to negative after adding the interaction term between café-au-lait spots and subcutaneous neurofibromas, indicating a positive three-way interaction. Café-au-lait spots, intertriginous freckles and subcutaneous neurofibromas all involve cells derived from the embryonic neural crest [Weston et al. 1981]. This is consistent with the suggestion that NF1 is a neurocristopathy [Huson and Hughes 1994] but does not explain why other neural crest-derived tissues, such as the

sympathetic ganglia, thyroid C-cells, and parathyroids, are rarely involved in NF1. Moreover, many features of NF1, such as learning disabilities, dysplastic scoliosis, and tibial pseudarthrosis, do not appear to be abnormalities of neural-crest derived tissues.

The common thread between optic glioma, other neoplasms and macrocephaly could be glial hyperplasia resulting from haploinsuffiency of neurofibromin. Most of the other neoplasms in our patients involve the central nervous system and most of these are gliomas [Friedman and Birch 1997a]. Patients with hydrocephalus and plexiform neurofibromas on the head were excluded from the analyses of head circumference, so enlargement of the head in the remaining patients must be due to enlargement of the scalp, skull or brain. In NF1, enlargement of the brain is the likely cause [Huson 1994; Riccardi 1992]. Gutmann et al. [1999] have directly demonstrated an effect of *NF1* haploinsufficiency on glial cell proliferation.

The pathogenic basis for the association we observed between pseudarthrosis and other neoplasms is not well understood.

While these models are accurate descriptors of feature occurrence, they cannot be used to predict who will get what features. The NFDB data is largely cross-sectional, with 74% of the subjects seen only once. The MANF1 is exclusively cross-sectional. A fitted logistic regressive model can be used to predict the risk for an individual developing a particular feature in follow-up studies, but not in cross-sectional studies such as this one [Kleinbaum 1992]. Currently available longitudinal clinical data in NF1 are too limited in number of subjects and duration of study for this purpose; large-scale longitudinal studies of the natural history of NF1 would be necessary to develop predictive models.

Phenotypic studies of affected relatives can determine the importance of familial and genetic factors in the development of these common NF1 features. Family studies on NFDB patients may differentiate between the different familial mechanisms that could be contributing to NF1 expressivity.

REFERENCES

Allanson JE, Upadhyaya M, Watson GH, et al. 1991. Watson syndrome: is it a subtype of type 1 neurofibromatosis? J Med Genet 28:752-6.

Burger PC, Scheithauer BW. 1994. Tumors of the central nervous system. Atlas of tumor pathology. Third series, fascicle 10. Armed Forces Institute of Pathology, Washington.

Caronti B, Buttarelli FR, Giustini S, Calderaro C, Calandriello L, Calvieri S, Palladini G .1998. Serum mitogenic activity on in vitro glial cells in neurofibromatosis type 1. Brain Res 793:21-8.

DeBella K, Szudek J, Friedman JM. 2000. Use of National Institutes of Health criteria for diagnosis of neurofibromatosis 1 in children. Pediatrics 105:608-614.

Easton DF, Ponder MA, Huson SM, Ponder BA. 1993. An analysis of variation in expression of neurofibromatosis type 1 (NF1): evidence for modifying genes. Am J Hum Genet 53:305-13.

Fitzpatrick TB. 1981. Melanin synthesis pathways in the pathogenesis of neurofibromatosis. Adv Neurol 29:209-11.

Friedman JM. 1999. Clinical and epidemiological features. In: Friedman JM, Gutmann DH, MacCollin M, Riccardi V. Neurofibromatosis: phenotype, natural history, and pathogenesis. 3rd ed. Johns Hopkins University Press, Baltimore, pp 29-86.

Friedman JM, Birch P. 1997. Type 1 neurofibromatosis: a descriptive analysis of the disorder in 1,728 patients. Am J Med Genet 70:138-43.

Friedman JM, Birch P. 1997b. An association between optic glioma and other tumours of the central nervous system in neurofibromatosis type 1. Neuropediatrics 282:131-2.

Gutmann DH, Aylsworth A, Carey JC, et al. 1997. The diagnostic evaluation and multidisciplinary management of neurofibromatosis 1 and neurofibromatosis 2. JAMA 278:51-57.

Gutmann DH, Loehr A, Zhang Y, Kim J, Henkemeyer M, Cashen A. 1999. Haploinsufficiency for the neurofibromatosis 1 (NF1) tumor suppressor results in increased astrocyte proliferation. Oncogene 18:4450-9.

Hamill PV, Drizd TA, Johnson CL, Reed RB, Roche AF. 1977. NCHS growth curves for children birth-18 years, U. S. 1967-73. Vital and health statistics. Series 11, No. 165. DHHS Pub. No. (PHS) 78-1650. U.S. Government Printing Office, Washington.

Harkin JC, Reed RJ. 1969. Tumors of the peripheral nervous system. Atlas of tumor pathology. Second Series, Fascicle 3. Armed Forces Institute of Pathology, Washington.

Hosmer DW, Lemeshow S. 1989. Applied logistic regression. John Wiley and Sons, Toronto.

Huson SM. 1994. Neurofibromatosis 1: A clinical and genetic overview. In: Huson SM and Hughes RAC (eds) The neurofibromatoses: A pathogenic and clinical overview. Chapman and Hall, New York, pp 160-204.

Huson SM, Hughes RAC (eds). 1994. The neurofibromatoses: a pathogenetic and clinical overview. Chapman & Hall, New York.

Kleinbaum DG. 1992. Logistic regression: a self-learning text. Spinger-Verlag, New York.

McGaughran JM, Harris DI, Donnai D, et al. 1999. A clinical study of type 1 neurofibromatosis in north west England. J Med Genet 36:197-203.

Perry HD, Font RL. 1982. Iris nodules in von Recklinghausen's Neurofibromatosis. Electron microscopic confirmation of their melanocytic origin. Arch Ophthalmol 100:1635-40.

Pietruschka G. 1961. Zur symptomatic der neurofibromatosis multiplex nach von Recklinghausen im Bereich des Sehorgans. Med Bild 4:8-11.

Riccardi VM. 1992. Neurofibromatosis: phenotype, natural history and pathogenesis. 2nd ed. Johns Hopkins University Press, Baltimore.

SAS Institute. 1996. SAS release 6.12, Cary, NC.

Sawada S, Florell S, Purandare SM, et al. 1996. Identification of NF1 mutations in both alleles of a dermal neurofibroma. Nat Genet 14:110-2.

Serra E, Puig S, Otero D, et al. 1997. Confirmation of a double-hit model for the NF1 gene in benign neurofibromas. Am J Hum Genet 61:512-9.

Szudek J, Birch P, Riccardi VM, Evans DG, Friedman JM. Associations of clinical features in neurofibromatosis 1 (NF1). Genet Epidemiol (in press).

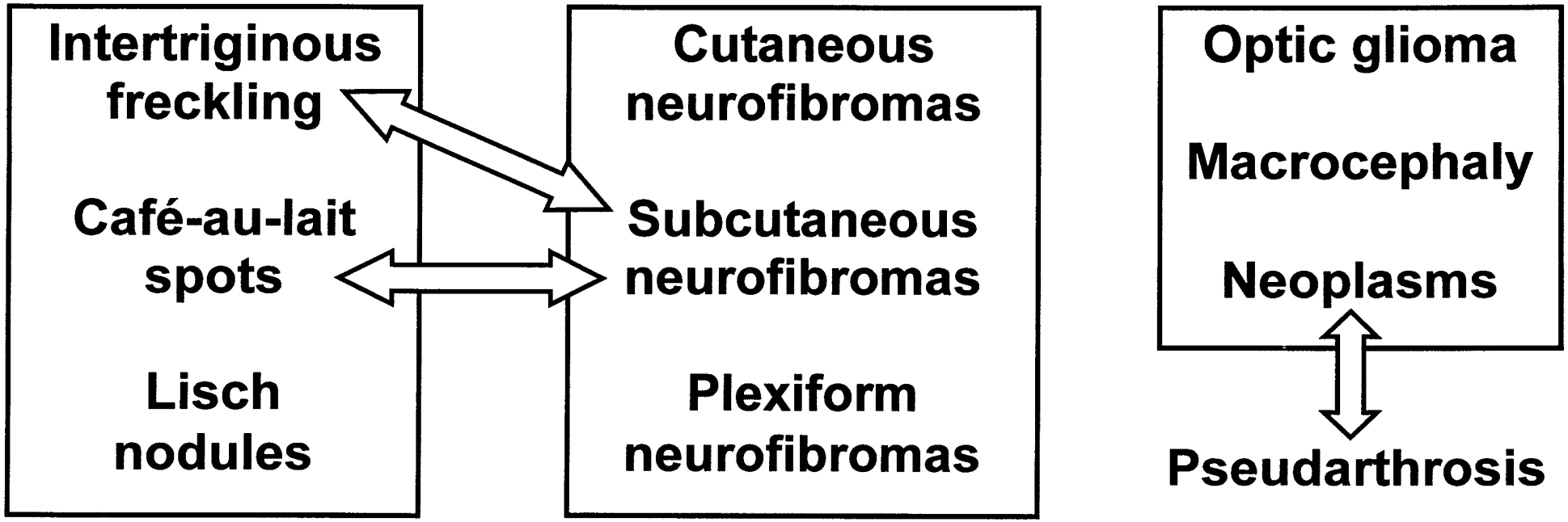
Upadhyaya M, Sarfarazi M, Huson, S et al. 1990. Linkage of Watson syndrome to chromosome 17 Markers. J Med Genet 27:209.

Weston JA. 1981. The regulation of normal and abnormal neural crest cell development. Adv Neurol 29:77-95.

Zehavi C, Romano A, Goodman RM. 1986. Iris (Lisch) nodules in neurofibromatosis. Clin Genet 29:51-55.

FIGURE LEGEND

Figure 1: Proposed grouping of NF1 features, based on the odds ratios in Table III. Features enclosed by a box or connected by an arrow are important variables in each other's models.


Intertriginous freckling
Café-au-lait spots
Lisch nodules
Cutaneous neurofibromas
Subcutaneous neurofibromas
Plexiform neurofibromas
Optic glioma
Macrocephaly
Neoplasms
Pseudarthrosis

Logistic regressive models of associations among neurofibromatosis 1 (NF1) features. *J. Szudek[1], H. Joe[2], J.M. Friedman[1].* 1) Dept Medical Genetics; 2) Dept Statistics; Univ British Columbia, Vancouver, BC, Canada.

Most severe features of NF1 are uncommon but unpredictable because of extreme clinical variability. We have previously shown that associations exist between some pairs of NF1 features. We now extend the analysis to associations of multiple different features.

Twelve features were analyzed in data from 2,797 probands in the National Neurofibromatosis Foundation International Database: freckling, discrete and plexiform neurofibromas, Lisch nodules, optic gliomas, seizures, learning disability, pseudarthrosis, scoliosis, macrocephaly, short stature, and neoplasms. Models were developed using half of the data, then tested on the remaining data.

Each of the 12 features was set as the output variable in a bivariate logistic regressive model to screen the remaining 11 features as potential main effects, with age as a continuous covariate. Significant features were entered in multivariate models, the importance of each variable reassessed, and significant variables used to revise each model. Interaction terms among the explanatory variables were then considered and significant terms added. Only models that fit both subsets by the Hosmer and Lemeshow statistic were considered valid.

The results reveal several associations. For example, Lisch nodules are 8.7 times more frequent in subjects with freckling, discrete neurofibromas and neoplasms than in subjects lacking these features. Optic glioma occurs 14 times more often in subjects with plexiform neurofibromas and neoplasms than in subjects who lack these features. Similar associations have been observed for several other features.

These findings demonstrate that some NF1 patients are far more likely than others to have certain features of the disorder. This suggests that fundamental biological differences exist between subjects who differ by particular constellations of features. Logistic regressive models may be useful for determining age- and sex-specific risks for complications of NF1 in clinically defined sub-groups of NF1 patients.

ANALYSIS OF LOCAL AND FAMILIAL FACTORS IN NEUROFIBROMATOSIS 1 LESIONS

Word count: 2,827

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ABSTRACT

Background: The defining feature of neurofibromatosis 1 (NF1) is the neurofibroma, a complex benign tumor arising from peripheral nerve sheaths. The number of neurofibromas in NF1 patients increases with age and is highly variable; the cause of this variability is unknown. Most young children with NF1 do not have any neurofibromas, but almost all have multiple café-au-lait spots.

Objective: To test the hypothesis that development of neurofibromas and café-au-lait spots may be influenced by local and familial factors.

Setting: Survey of records from patients seen at a specialized NF1 clinic between 1979 and 1995.

Design and Patients: The presence of one or more café-au-lait spots, one or more dermal discrete neurofibromas, and one or more diffuse plexiform neurofibromas was recorded for each of ten divisions of the body surface in 547 NF1 patients, including 117 affected individuals in 52 families. We tested for local associations between the presence of diffuse plexiform neurofibromas and dermal discrete neurofibromas in individual body segments of each NF1 patient. We stratified simultaneously by the body segment being considered and by the total number body segments with one or more dermal discrete neurofibromas. Local associations between café-au-lait spots and dermal discrete neurofibromas and between café-au-lait spots and plexiform neurofibromas were also analyzed in the same manner. We also tested for correlations in the number of body segments affected with each of the three lesions in members of the same family, while controlling for age.

Results: We found no overall association between the occurrence of dermal discrete and diffuse plexiform neurofibromas, café-au-lait spots and dermal discrete neurofibromas, or café-au-lait spots and plexiform neurofibromas in the same body segments. We found the correlation among relatives in the number of body segments affected with one or more lesions to be 0.37 (95% confidence interval (CI)=0.15,0.55) for dermal discrete neurofibromas, 0.35 (95% CI=0.15,0.57) for plexiform neurofibromas and 0.45 (95% CI=0.18,0.71) for café-au-lait spots.

Conclusions: The development of dermal discrete neurofibromas, plexiform neurofibromas, and café-au-lait spots in NF1 patients are each spatially independent but influenced by familial factors.

INTRODUCTION

Neurofibromatosis 1 (NF1) is an autosomal dominant condition affecting 1 in 3000 individuals. Its defining feature is the neurofibroma: a complex benign tumor arising in the fascicles of peripheral nerves. Histologically, a local increase in endoneurial matrix of the fascicle is accompanied by a thickened perineurium, Schwann cells that increase in size and number (Harkin and Reed 1969), and an increased number of mast cells and fibroblasts (Giorno et al. 1989). Dermal discrete neurofibromas are confined to a single fascicle within a nerve, occur in most NF1 patients and tend to increase in number with age (Riccardi 1982b; Burger and Scheithauer 1994). Diffuse plexiform neurofibromas involve multiple fascicles and patients who present with them usually do so during childhood. Some diffuse and most deep nodular plexiform neurofibromas are asymptomatic and not apparent on surface exam (Riccardi 1992).

Café-au-lait spots are another cardinal pathologic feature of NF1. They are present at birth in almost all NF1 patients and their number and size tend to increase during the first decade of life (Riccardi 1982a). This makes them particularly useful in making the diagnosis of NF1 (DeBella et al. 2000). They are typically 10 to 30 mm in diameter and can occur anywhere on the skin except the scalp, eyebrows, palms and soles (Riccardi 1992). The basal layer of the epidermis in a café-au-lait spot contains melanocytes with abnormally large melanosomes – specialised intracellular organelles for melanin synthesis (Fitzpatrick 1981). As the Schwann cells in neurofibromas are derived from the neural crest (Johnston et al. 1981), so are the melanocytes in café-au-lait spots (Bolande 1974). Whereas neurofibromas can be a source of pain and morbidity, café-au-lait spots cause only cosmetic problems (Gutmann et al. 1997). The number and

location of neurofibromas and café-au-lait spots are highly variable, even among NF1 patients of similar age. The cause of this variability is unknown.

It has been hypothesised that histamine or other products secreted by mast cells may influence the growth of neurofibromas (Giorno et al. 1989; Riccardi 1992). A neurofibroma that contains an excess of mast cells could stimulate not only its own growth, but that of other neurofibromas nearby. Secreted factor may also be triggered by local trauma. Freckles and other areas of hyperpigmentation tend to occur in skin folds, presumably due to local environmental factors (Fitzpatrick 1981; Riccardi 1992). Dermal discrete neurofibromas may arise in an area that has been injured (Riccardi 1990).

We have shown previously that NF1 patients with dermal discrete neurofibromas are more likely also to have diffuse plexiform neurofibromas and café-au-lait spots than NF1 patients without dermal discrete neurofibromas (Szudek et al. 2000). Several other NF1 clinical features were found to be associated between affected parents and children. Here we test the hypotheses that the development of these lesions may be influenced by local or familial factors.

PATIENTS AND METHODS

Patients. 547 NF1 patients, including 117 affected individuals in 52 families, were selected from the NF Institute database (Riccardi 1992). All of these patients were evaluated between 1979 and 1995 by Dr. Vincent Riccardi and all met the NIH diagnostic criteria for NF1 at the time of examination (NIH 1988; Gutmann et al. 1997). For each patient, the presence of one or more café-au-lait spots, one or more dermal discrete neurofibromas, and one or more diffuse plexiform neurofibromas was recorded

for each of the ten divisions of the body surface shown in Figure 1.

Analysis of local effect. We used two-layered Mantel-Haenszel tests to look for local associations between the presence of diffuse plexiform neurofibromas and dermal discrete neurofibromas in individual body segments of each NF1 patient (SPSS 1998). We stratified simultaneously by the body segment being considered and by the total number of body segments with one or more dermal discrete neurofibromas (a categorical variable with range 0 to 10). This stratification was used to adjust for the fact that an NF1 patient who has a larger total number of body segments with one or more neurofibromas is more likely to have at least one neurofibroma in any particular segment than an NF1 patient who has fewer total body segments affected. Confidence intervals for the summary odds-ratios were obtained using a jack-knife based on 20 different subgroups –sufficient to get stability in the estimate (Miller 1974). Homogeneity was assessed using the Breslow-Day test (SPSS 1998). Local associations between café-au-lait spots and dermal discrete neurofibromas and between café-au-lait spots and plexiform neurofibromas were also analyzed in this manner.

Skin surface area. The body divisions used in this study cover varying amounts of skin surface area, so we checked for an association between segment surface area and the presence of one or more dermal discrete neurofibroma. Using logistic regression, we set the segment area as the independent variable and the presence or absence of dermal discrete neurofibromas as the dependent variable. We also checked for an association between surface area and prevalence of diffuse plexiform neurofibromas. Since the

median age of our patients was 12 years, we approximated the surface area percentages of the body divisions by taking the mean of the values cited for children and for adults (Palehorse 1997). The proportions of total surface area assigned to each body segment were: head=9%, neck=3%, right upper torso=9%, left upper torso=9%, right lower torso=9%, left lower torso=9%, right arm=9%, left arm=9%, right leg=17%, and left leg=17%.

Total number of dermal discrete neurofibromas. In addition to the data on whether each body segment was affected by one or more dermal discrete neurofibromas, complete counts of dermal discrete neurofibromas were available for 44 of the patients. Counts of dermal discrete neurofibromas ranged from none to several hundred and appeared to increase logarithmically with the number of affected segments. We log-transformed the counts of dermal discrete neurofibromas and plotted them against number of divisions with one or more dermal discrete neurofibromas to test whether the number of body segments with one or more dermal discrete neurofibromas provided a good representation of the total number of dermal discrete neurofibromas in an individual. We used linear regression to quantify this relationship (SPSS 1998). Counts of total number of café-au-lait spots were not made, and few subjects had more than one plexiform neurofibroma, so these variables were not analyzed in this manner.

Familial analysis. For the familial analysis, we stratified subjects into 5-year age intervals, calculated the deciles for the total number of segments affected with dermal discrete neurofibromas in each stratum, and then ranked each subject by decile for the

stratum in which he or she lay. We then used random effects models to obtain maximum likelihood estimates and confidence intervals for intrafamilial correlations for decile (Spjotvoll 1967; Donner et al. 1989). Café-au-lait spots and plexiform neurofibromas were also analyzed in the same manner.

RESULTS

We studied the distribution of café-au-lait spots, dermal discrete neurofibromas, and diffuse plexiform neurofibromas in 10 segments of the body surface (Figure 1) of each of 547 patients with NF1. Fifty-three percent (n=385) of the subjects were female, and 47% (n=344) male; 77% (n=426) were White, 12% (n=) were Hispanic, 8% (n=44) were Black and 1% (n=8) were of other or mixed origin. Mean age was 17 years, and median age was 12 years.

Lesion frequency by body segment. 210 patients had no dermal discrete neurofibromas in any segment. 4 patients had no café-au-lait spots in any segment. 331 patients had no plexiform neurofibromas in any segment. Table 1 shows the frequency of these lesions in each of the 10 body segments. Dermal discrete and plexiform neurofibromas occurred with similar frequencies in all 10 body segments. Café-au-lait spots occurred with similar frequencies in all body segments except the head, where they were less frequent.

No associations between lesion types within individual body segments. Table 2 shows the 10 body segments examined and the pair-wise odds-ratio between neurofibromatosis

lesions for each segment. No association was observed between the occurrence of dermal discrete and diffuse plexiform neurofibromas in the same body segment. The summary odds-ratio was 1.20 (95% CI=0.81,1.79). There was no evidence for heterogeneity across body divisions (p=0.37). Similarly, there was no association between the presence of café-au-lait spots and either dermal discrete or diffuse plexiform neurofibromas within a single body segment. The summary odds-ratios were 1.26 (95% CI=0.82,1.93) for café-au-lait spots and dermal discrete neurofibromas and 1.25 (95% CI=0.74,2.12) for café-au-lait spots and plexiform neurofibromas. There was no evidence of heterogeneity across body divisions for the occurrence of plexiform neurofibromas with café-au-lait spots (p=0.52), but there was significant (p=0.03) heterogeneity in the occurrence of dermal discrete neurofibromas and café-au-lait spots, with a positive association seen in the neck (odds ratio=2.94; 95% CI=1.20,7.20).

Log-linear relationship between segment size and number of dermal discrete neurofibromas. We observed no association between the relative size of the body surface area in a segment and the presence of one or more dermal discrete neurofibromas (p=0.74) or of a diffuse plexiform neurofibroma (p=0.17). The number of body segments affected with one or more dermal discrete neurofibromas was strongly correlated with the total number of dermal discrete neurofibromas in 44 NF1 patients in whom both total counts and data on the number of affected body segments were available (r=0.95, $p<0.001$). The relationship is log linear (Figure 2); the regression equation is log(total number of neurofibromas +1) = 0.23*(number of segments affected) + 0.014.

All three lesions are correlated among relatives with NF1. We estimated intrafamilial correlations in the age-adjusted number of body segments that included one or more dermal discrete neurofibromas, one or more café-au-lait spots, or one or more plexiform neurofibromas in 117 affected members of 52 families. We found significant intrafamilial correlations in the number of body segments affected by each of these clinical features. The correlation among relatives in the age-adjusted number of body segments with one or more dermal discrete neurofibromas was 0.37 (95% CI=0.15,0.55). The correlation among relatives in the age-adjusted number of body segments with one or more plexiform neurofibromas was 0.35 (95% CI=0.15,0.57). The correlation among relatives in the age-adjusted number of body segments with one or more café-au-lait spots was 0.45 (95% CI=0.18,0.71).

DISCUSSION

Lesions in body segments of individual patients. The number of body segments affected by one or more dermal discrete neurofibromas appears to provide a good measure of how severely each of these NF1 patients was affected by this disease feature. We found a very high correlation with the number of body segments affected in 44 patients in whom counts of the total number of dermal discrete neurofibromas were available. It seems likely that a similar relationship exists between the number of body segments affected with café-au-lait spots or plexiform neurofibromas and the severity of each of these disease features, but count data were not available in this study to

demonstrate this.

The subjects were referred to a specialized clinic, so we were concerned that they are more severely affected than the NF1 patient at large. The frequencies of dermal discrete neurofibromas, plexiform neurofibromas and café-au-lait spots are comparable to those from another large database (Friedman and Birch 1997) and population based studies (Samuelsson and Axelsson 1981; Huson et al. 1989; McGaughran et al. 1999). All of the subjects in this study were examined by the same clinician, ruling out the bias inherent in using multiple examiners. Body segment data was collected over a single visit for each patient, but the collection period lasted 16 years. We expect this to make the data less consistent than if it were gathered over a shorter period of time.

We have shown previously that individuals with diffuse plexiform neurofibromas are more likely also to have dermal discrete neurofibromas (Szudek et al. 2000), but this association did not take into account the location or number of these lesions. The current study is the first to examine this association within body divisions. Since virtually all diffuse plexiform neurofibromas are of congenital origin (Riccardi 1992), we wanted to find out if they influence the subsequent development of dermal discrete neurofibromas. Our findings suggest that the occurrence of dermal discrete neurofibromas in NF1 patients is not influenced by the local presence of a diffuse plexiform neurofibroma. In fact, we found that all three of the lesions studied (café-au-lait spots, dermal discrete neurofibromas, and plexiform neurofibromas) occurred independently of each another in almost all of the body segments analyzed (Table 2).

Mast cells have been implicated in the pathogenesis of plexiform neurofibromas (Giorno et al. 1989). It has been hypothesized that histamine or other products secreted

by these cells may influence the growth of neurofibromas, either the plexiform neurofibroma itself or other dermal discrete lesions(Riccardi 1992). This effect would presumably be strongest near the original plexiform neurofibroma. Our results argue against the hypothesis that factors secreted by a diffuse plexiform neurofibroma stimulate the development of nearby dermal discrete neurofibromas.

Local trauma has also been implicated in the pathogenesis of neurofibromatosis lesions. Dermal discrete neurofibromas may arise in an area that has been injured (Riccardi 1990). Freckles and other areas of hyperpigmentation tend to occur in skin folds, presumably due to local environmental factors(Fitzpatrick 1981; Riccardi 1992). We found a significant association between café-au-lait spots and dermal discrete neurofibromas only in the neck segment (Table 2). However, café-au-lait spots were found on all segments except the head and neck in almost all patients (Table 1), making it hard to detect significant associations in most segments. The result could also be due to chance alone, since many different associations were calculated. One pathological reason the neck might be affected by both lesions is recurrent minor trauma to the skin associated with flexion, extension, and rotation of the head (Riccardi 1990). Clearly, however, other factors are also involved in the pathogenesis of café-au-lait spots and neurofibromas, as indicated by the familial correlations we observed for the age-adjusted number of body segments affected by each of the three lesions studied.

Familial correlations. The significant intrafamilial correlations we found are consistent with other evidence that familial factors contribute to the development of dermal discrete neurofibromas and café-au-lait spots in patients with NF1 (Easton et al. 1993; Szudek et

al. 1998). The number of familial patients and the prevalences of all three lesions were similar between the three study groups (Riccardi 1992; Easton et al. 1993; Friedman and Birch 1997). The present study found a similar correlation for café-au-lait spots but higher correlations for dermal discrete neurofibromas than Easton et al. We also found a significant correlation for plexiform neurofibromas, whereas Easton et al. only analyzed this feature as a binary trait and found no familial association. Our use of 10 body segments allows for more precise stratification than Easton's 5 categories and may account partly for the differences.

The genetic basis for these familial associations has not been determined, but contributing factors may include effects of the mutant *NF1* allele itself, effects of the normal *NF1* allele, or modifying effects of other loci. The moderate magnitudes of the intrafamilial correlation coefficients show that family history alone is insufficient to predict the degree to which a patient will be affected with these lesions. Other elements must also be involved.

Our results are consistent with the possibility that different pathogenic mechanisms are responsible for the three lesions studied. Dermal discrete neurofibromas are composed of Schwann cells, fibroblasts, mast cells and axons(Korf 1999). Schwann cells are thought to play a major role in tumourgenesis since they have been found to undergo loss of heterozygosity at the *NF1* locus, while other neurofibroma cells have not(Kluwe et al. 1999; Rutkowski et al. 2000). Although the mechanism by which loss of *NF1* function leads to neurofibromas is unknown, Schwann cells that give rise to dermal discrete neurofibromas migrate during embryonic development from the neural crest (Johnston et al. 1981) and arise through a process of neoplasia, at least in many cases

(Stark et al. 1995).

Although plexiform neurofibromas also contain Schwann cells, fibroblasts, mast cells and axons, they are congenital and have more characterisitcs of dysplasia than do dermal discrete neurofibromas(Riccardi 1992). Plexiform neurofibromas usually have extensive vascularisation, involve many different tissues and can spread to distort adjacent tissues(Korf 1999).

Chimeric mice composed in part of *Nf1*$^{-/-}$ cells develop plexiform neurofibromas but not dermal discrete neurofibromas(Cichowski et al. 1999; Vogel et al. 1999). On the other hand, transgenic mice expressing the human T-lymphotropic virus type 1 *tax* gene develop dermal discrete neurofibromas(Hinrichs et al. 1987; Nerenberg et al. 1987; Green et al. 1992; Feigenbaum et al. 1996). Although *Nf1* repression by *tax* occurs in the absence of mutation at the *Nf1* locus, these observations suggests that plexiform and discrete neurofibromas can arise by pathways that are independent, at least in mice. Like neurofibromas, café-au-lait spots contain neural crest derived cells, but these are melanocytes with abnormally large pigment particles (Fitzpatrick 1981) rather than Schwann cells, as in neurofibromas. Some human families with *NF1* mutations develop café-au-lait spots but no tumours, consistent with different pathogenic factors being involved in the development of neurofibromas (Abeliovich et al. 1995).

In summary, our findings are consistent with multiple factors being involved in the pathogenesis of both plexiform and dermal discrete neurofibromas as well as of café-au-lait spots. Some of these factors appear to be genetic, but others are not. Although some of the pathogenic factors may be shared among these three lesions, others appear to differ.

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REFERENCES

Abeliovich D, Gelman-Kohan Z, Silverstein S, Lerer I, Chemke J, Merlin S, Zlotogora J (1995) Familial café au lait spots: a variant of neurofibromatosis type 1. J Med Genet 32:985-986

Bolande R (1974) The neurocristopathies: A unifying concept of disease arising in neural crest development. Hum Pathol 5:409-429

Burger P, Scheithauer B (1994) Tumors of the central nervous system Atlas of tumor pathology. Vol. Fascicle 10. Armed Forces Institute of Pathology, Washington, DC

Cichowski K, Shih TS, Schmitt E, Santiago S, Reilly K, McLaughlin ME, Bronson RT, Jacks T (1999) Mouse models of tumor development in neurofibromatosis type 1. Science 286:2172-6

DeBella K, Szudek J, Friedman JM (2000) Use of the national institutes of health criteria for diagnosis of neurofibromatosis 1 in children. Pediatrics 105:608-14

Donner A, Wells G, Eliasziw M (1989) On two approximations to the F-distribution: application to testing for intraclass correlation in family studies. Canadian Journal of Statistics 17:209-215

Easton D, Ponder M, Huson S, Ponder B (1993) An analysis of variation in expression of neurofibromatosis (NF) type I (NFI): Evidence for modifying genes. Am J Hum Genet 53:305-313

Feigenbaum L, Fujita K, Collins FS, Jay G (1996) Repression of the NF1 gene by Tax may expain the development of neurofibromas in human T-lymphotropic virus type 1 transgenic mice. J Virol 70:3280-5

Fitzpatrick TB (1981) Melanin synthesis pathways in the pathogenesis of neurofibromatosis. Adv Neurol 29:209-11

Friedman J, Birch P (1997) Type 1 neurofibromatosis: A descriptive analysis of the disorder in 1,728 patients. Am J Med Genet 70:138-143

Giorno R, Lieber J, Claman HN (1989) Ultrastructural evidence for mast cell activation in a case of neurofibromatosis. Neurofibromatosis 2:35-41

Green JE, Baird AM, Hinrichs SH, Klintworth GK, Jay G (1992) Adrenal medullary tumors and iris proliferation in a transgenic mouse model of neurofibromatosis. Am J Pathol 140:1401-10

Gutmann D, Aylsworth A, Carey J, Korf B, Marks J, Pyeritz R, Rubenstein A, Viskochil D (1997) The diagnostic evaluation and multidisciplinary management of neurofibromatosis 1 and neurofibromatosis 2. JAMA 278:51-57

Harkin J, Reed R (1969) Tumors of the peripheral nervous system Atlas of Tumor Pathology. Armed Forces Institute of Pathology, Washington, DC, pp 67-100

Hinrichs SH, Nerenberg M, Reynolds RK, Khoury G, Jay G (1987) A transgenic mouse model for human neurofibromatosis. Science 237:1340-3

Huson S, Compston D, Clark P, Harper P (1989) A genetic study of von Recklinghausen neurofibromatosis in south east Wales: I. Prevalence, fitness, mutation rate, and effect of parental transmission on severity. J Med Genet 26:704-711

Johnston MC, Vig KW, Ambrose LJ (1981) Neurocristopathy as a unifying concept: clinical correlations. Adv Neurol 29:97-104

Kluwe L, Friedrich R, Mautner VF (1999) Loss of NF1 allele in Schwann cells but not in fibroblasts derived from an NF1-associated neurofibroma. Genes Chromosomes Cancer 24:283-5

Korf B (1999) Neurofibromas and malignant tumors of the peripheral nerve sheath. In: Friedman J, Gutmann D, MacCollin M, Riccardi V (eds) Neurofibromatosis : phenotype, natural history, and pathogenesis. Johns Hopkins University Press, Baltimore, pp 142-161

McGaughran JM, Harris DI, Donnai D, Teare D, MacLeod R, Westerbeek R, Kingston H, Super M, Harris R, Evans DG (1999) A clinical study of type 1 neurofibromatosis in north west England. J Med Genet 36:197-203

Miller R (1974) The jackknife - a review. Biometrika 61:1-15

Nerenberg M, Hinrichs SH, Reynolds RK, Khoury G, Jay G (1987) The tat gene of human T-lymphotropic virus type 1 induces mesenchymal tumors in transgenic mice. Science 237:1324-9

NIH (1988) Neurofibromatosis: Conference statement. National Institutes of Health Consensus Development Conference. Arch Neurol 45:575-8

Palehorse (1997) Burns: Identification, Types and Treatment. JRH Enterprises

Riccardi V (1982a) The multiple forms of neurofibromatosis. Pediatr Rev 3:292-298

Riccardi V (1982b) Neurofibromatosis: Clinical heterogeneity. Curr Probl Cancer 7(2):1-34

Riccardi V (1990) The potential role of trauma and mast cells in the pathogenesis of neurofibromas. In: Ishibashi Y, Hori Y (eds) Tuberous sclerosis and neurofibromatosis : epidemiology, pathophysiology, biology, and management. Elsevier, Amsterdam, pp 167-190

Riccardi V (1992) Neurofibromatosis: Phenotype, natural history, and pathogenesis. The Johns Hopkins University Press, Baltimore

Rutkowski JL, Wu K, Gutmann DH, Boyer PJ, Legius E (2000) Genetic and cellular defects contributing to benign tumor formation in neurofibromatosis type 1. Hum Mol Genet 9:1059-66

Samuelsson B, Axelsson R (1981) Neurofibromatosis: A clinical and genetic study of 96 cases in Gothenburg, Sweden. Acta Dermatovenereolog Suppl 95:67-71

Spjotvoll E (1967) Optimum invariant tests in unbalanced variance component models. Ann Math Statist 38: 422-428

SPSS (1998) SPSS for Windows, Chicago

Stark M, Assum G, Krone W (1995) Single-cell PCR performed with neurofibroma Schwann cells reveals the presence of both alleles of the neurofibromatosis type 1 (NF1) gene. Hum Genet 96:619-23

Szudek J, Birch P, Riccardi VM, Evans DG, Friedman JM (2000) Associations of clinical features in neurofibromatosis 1 (NF1). Genet Epidemiol 19:429-39

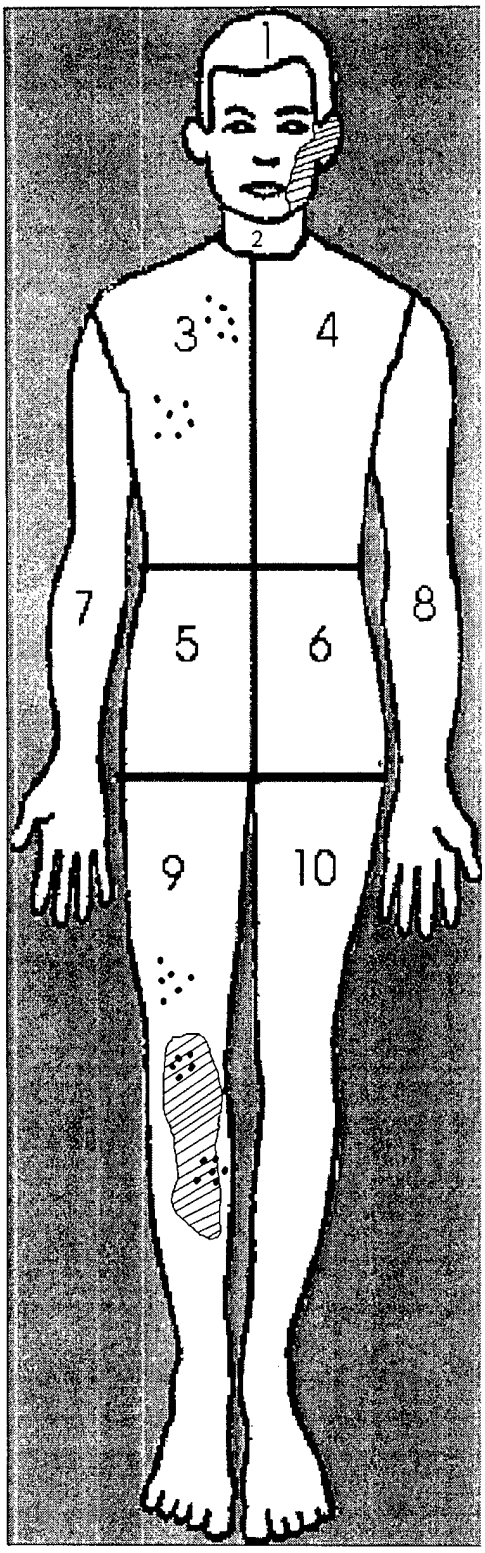
Szudek J, Evans D, Friedman J (1998) Logistic regresssive models of neurofibromatosis 1 (NF1) clinical features American Society of Human Genetics Annual Meeting. Am J Hum Genet, Baltimore

Vogel KS, Klesse LJ, Velasco-Miguel S, Meyers K, Rushing EJ, Parada LF (1999) Mouse tumor model for neurofibromatosis type 1. Science 286:2176-9

FIGURE LEGEND

Figure 1: Body segment scheme used by Neurofibromatosis Institute Database

Figure 2: Correlation between the number of body segments affected with one or more dermal discrete neurofibromas and the total number of dermal discrete neurofibromas in 44 NF1 patients ($r=0.95$, $p<0.001$). Total neurofibroma counts and data on the number of affected body segments were available in all patients. The relationship is log linear; the regression equation is Log(total number of neurofibromas +1) = 0.23*(number of segments affected) + 0.014.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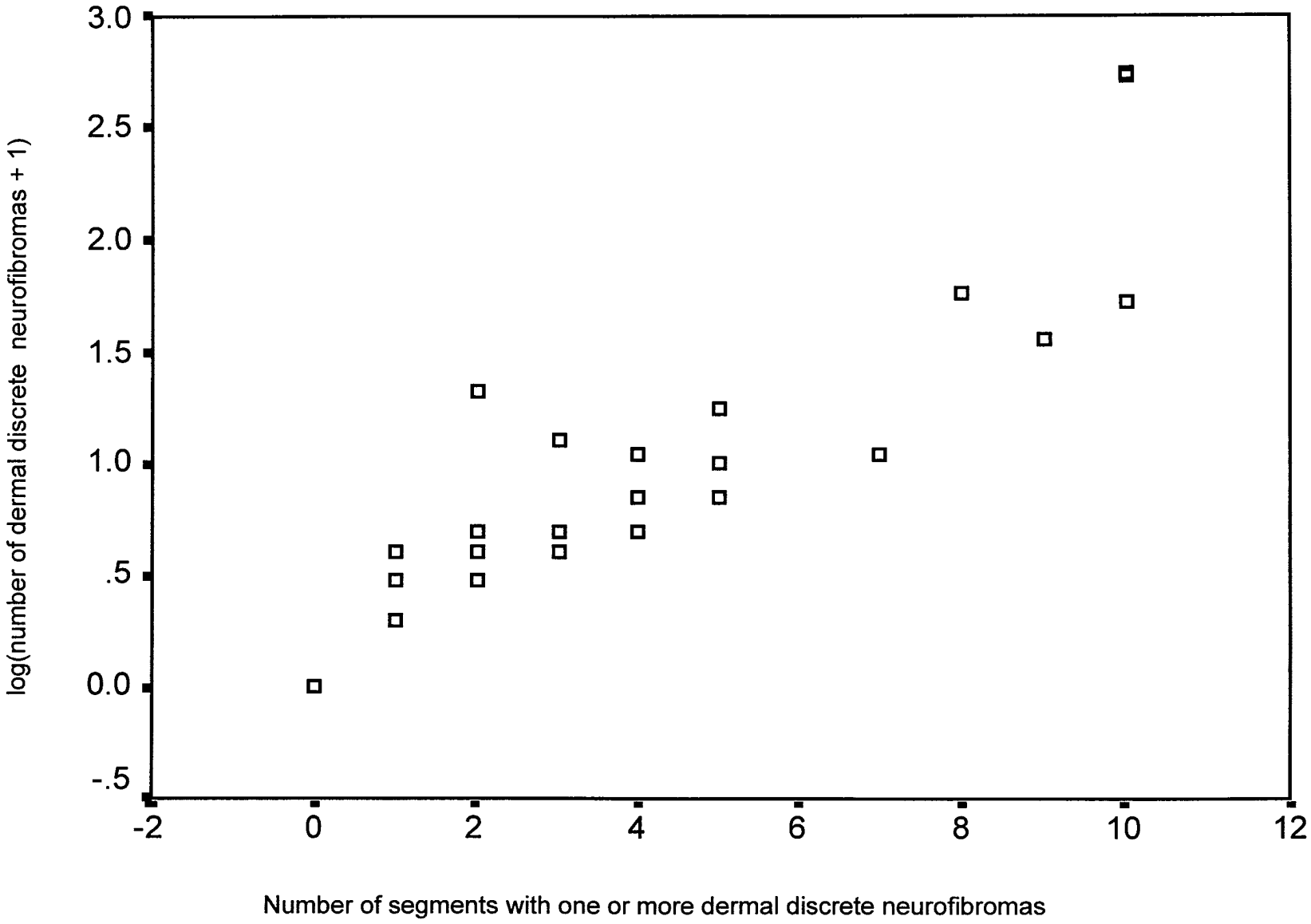
3.0
2.5
2.0
1.5
1.0
.5
0.0
-.5
log(number of dermal discrete neurofibromas + 1)
-2
0
2
4
6
8
10
12
Number of segments with one or more dermal discrete neurofibromas

Table 1: Number and percentage of patients with dermal discrete neurofibromas, diffuse plexiform neurofibromas and café-au-lait spots by body segment in 547 NF1 patients.

		Dermal Discrete Neurofibromas		Plexiform Neurofibromas		Café-au-lait spots	
Segment		**Total**	**(%)**	**Total**	**(%)**	**Total**	**(%)**
I	Head	179	(33%)	47	(8%)	101	(18%)
II	Neck	168	(31%)	29	(5%)	397	(73%)
III	Right Upper Torso	259	(47%)	32	(6%)	532	(97%)
IV	Left Upper Torso	258	(47%)	21	(4%)	531	(97%)
V	Right Lower Torso	285	(52%)	55	(10%)	537	(98%)
VI	Left Lower Torso	287	(52%)	41	(7%)	533	(97%)
VII	Right Arm	206	(38%)	21	(4%)	514	(94%)
VIII	Left Arm	208	(38%)	19	(3%)	511	(93%)
IX	Right Leg	219	(40%)	54	(10%)	527	(96%)
X	Left Leg	220	(40%)	45	(8%)	525	(96%)
Total		***337***	***(62%)***	***216***	***(39%)***	***543***	***(99%)***

Table 2: Associations between dermal discrete neurofibromas, diffuse plexiform neurofibromas and café-au-lait spots by body segment in 547 NF1 patients.

		Dermal Discrete NFs vs. Plexiform NFs		**Dermal Discrete NFs vs. Café-au-lait spots**		**Café-au-lait spots vs. Plexiform NFs**	
Segment		**Odds Ratio**	**(95% C.I.)**	**Odds Ratio**	**(95% C.I.)**	**Odds Ratio**	**(95% C.I.)**
I	Head	0.95	(0.34-2.68)	1.34	(0.67-2.67)	1.26	(0.60-2.65)
II	Neck	2.39	(0.51-11.20)	2.59	(1.23-5.47)	2.42	(0.71-8.24)
III	Right Upper Torso	0.83	(0.23-3.02)	0.26	(0.01-10.34)	-	-
IV	Left Upper Torso	0.39	(0.06-2.49)	0.12	(0.01-7.07)	1.29	(0.02-83.37)
V	Right Lower Torso	0.85	(0.32-2.24)	0.98	(0.01-84.41)	-	-
VI	Left Lower Torso	0.91	(0.35-2.36)	1.13	(0.19-6.94)	0.06	(0.01-0.99)
VII	Right Arm	1.17	(0.09-14.43)	1.91	(0.29-12.67)	-	-
VIII	Left Arm	1.01	(0.18-5.60)	0.91	(0.18-4.65)	0.22	(0.02-1.97)
IX	Right Leg	3.91	(1.02-15.06)	0.20	(0.04-1.15)	0.35	(0.05-2.34)
X	Left Leg	3.60	(0.99-13.08)	1.10	(0.24-5.00)	2.70	(0.17-44.12)
Summary		***1.20***	***(0.81-1.79)***	***1.36***	***(0.91-2.03)***	***1.25***	***(0.74-2.12)***

The development of cutaneous neurofibromas is influenced by familial and local factors in patients with Neurofibromatosis 1 (NF1). *C. Palmer[1], J. Szudek[1], H. Joe[2], V.M. Riccardi[3], J.M. Friedman[1].* 1) Dept Medical Genetics and; 2) Dept Statistics, University of British Columbia, Vancouver, Canada; 3) American Medical Consumers, Los Angeles, CA.

NF1 is an autosomal dominant condition affecting 1 in 3000 individuals. Its defining feature is the neurofibroma: a complex benign tumor arising from peripheral nerve sheaths. The number of cutaneous neurofibromas in NF1 patients increases with age and is highly variable; the cause of this variability is unknown. We tested the hypothesis that development of these lesions may be influenced by local and familial factors.

The presence or absence of 1 or more cafe au lait spots, 1 or more cutaneous neurofibromas, and 1 or more diffuse plexiform neurofibromas was recorded for each of ten divisions of the body surface in 768 NF1 patients, including 117 affected individuals in 52 families. We used a random effects model to obtain the maximum likelihood estimate and confidence interval of intrafamilial correlations in the number of body divisions affected with 1 or more cutaneous neurofibromas, while controlling for age. The correlation amongst first-degree relatives was ρ=0.30 (95% CI=0.078,0.52), in agreement with previous studies.

We used a Mantel-Haenszel test, stratified simultaneously by body division and number of body divisions with 1 or more cutaneous neurofibromas, to examine associations in the presence of diffuse plexiform neurofibromas and cutaneous neurofibromas in individual NF1 patients (n=630). Divisions that include a diffuse plexiform neurofibroma are twice as likely to have 1 or more cutaneous neurofibromas as well (summary odds ratio=2.02; 95% CI=1.28, 2.77). Odds ratios were not homogeneous across body divisions. No significant association was observed between the presence of cafe au lait spots and cutaneous neurofibromas in a body division in NF1 patients (n=584).

We conclude that the occurrence of cutaneous neurofibromas in NF1 patients is influenced by familial factors as well as by local factors.

ANALYSIS OF INTRA-FAMILIAL PHENOTYPIC VARIATION IN NEUROFIBROMATOSIS 1 (NF1)

Running Title: Intra-familial variation in neurofibromatosis 1

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ABSTRACT

The relationship of genetic factors to variable expressivity in neurofibromatosis 1 (NF1) is poorly understood. We examined familial aggregation of NF1 features among different classes of affected relatives. Clinical information was obtained from the National NF Foundation International Database on 913 affected individuals in 373 families with 2 or more members with NF1. We used multivariate probit and multivariate normal regression to measure the associations between various classes of relatives for each of 12 clinical features of NF1 while simultaneously adjusting for covariates including related features, age and gender.

Statistically significant associations among relatives were found for café-au-lait spots, intertriginous freckling, neurofibromas, Lisch nodules, head circumference and stature but not for optic glioma, other neoplasms, seizures or scoliosis. Three distinct patterns were observed among the associations for familial features when compared between 1st and 2nd degree relatives and between sib-sib and parent-child pairs: 1) Head circumference and stature had similar associations for all three relationships; 2) Lisch nodules and café-au-lait spots had greater associations between 1st degree relatives than between 2nd degree relatives; and 3) Subcutaneous neurofibromas, plexiform neurofibromas, café-au-lait spots, and intertriginous freckling had greater associations between sibs than between parents and children. In addition, Lisch nodules, subcutaneous neurofibromas and cutaneous neurofibromas had greater associations between affected fathers and children than between affected mothers and children. These familial patterns suggest that the mutant NF1 allele, unlinked modifying genes, and the normal NF1 allele may all be involved in the development of particular clinical features of NF1, but that the relative contributions vary for different features.

INTRODUCTION

Neurofibromatosis 1(NF1) is an autosomal dominant disease that affects about 1/3,500 people [Friedman, 1999]. NF1 can affect the skin, skeleton and nervous system and is characterized by highly variable expressivity [Riccardi, 1992]. Many disease features are progressive, but the rate of progression and the occurrence of serious manifestations vary greatly from one patient to another [Friedman and Riccardi, 1999]. This variability and the confounding effect of age have hindered efforts to characterize the relationship of genetic factors at the *NF1* locus or other loci to disease variability.

Mutation analysis of NF1 patients is difficult due to the large size of the *NF1* gene (335kb of genomic DNA) [Li, et al., 1995], the existence of multiple unlinked pseudogenes and the large variety of mutational lesions [Viskochil, 1999]. The most effective single method for mutation analysis is the protein truncation test, but it detects mutations in only about 2/3 of patients [Heim, et al., 1995, Park and Pivnick, 1998]. Messiaen et al. [Messiaen, et al., 2000] reported a mutation detection rate of greater than 90% in 67 NF1 probands, but this required a combination of protein truncation, heteroduplex, FISH, Southern blot and cytogenetic techniques.

More than 400 different constitutional *NF1* mutations have been reported [Messiaen, et al., 2000, Korf, 1999, Fahsold, et al., 2000]. In general, little evidence has been found of allele-phenotype correlations in NF1, although a more or less consistent phenotype occurs in association with deletions involving the entire *NF1* gene [Tonsgard, et al., 1997, Dorschner, et al., 2000]. Similar clinical features have been observed among affected members of a few families with the NF1 variants Watson syndrome [Allanson, et al., 1991], familial café-au-lait spots [Abeliovich, et al., 1995] or familial spinal neurofibromas [Pulst, et al., 1991, Poyhonen, et

al., 1997, Ars, et al., 1998]. This observation is consistent with an allele-phenotype correlation, but no consistent kind of *NF1* mutation has been found in families with these or other phenotypic variants. Affected members of a single family with typical NF1 often have quite different disease phenotypes, despite sharing an identical mutant *NF1* allele. Clearly, variation in the mutant *NF1* allele itself is insufficient to account for the variability of most disease features.

Mouse models for NF1 support the importance of other genetic factors in the development of disease features. Transgenic mice expressing the human T-lymphotropic virus type 1 *tax* gene develop dermal discrete neurofibromas and, occasionally, Lisch nodules [Hinrichs, et al., 1987, Nerenberg, et al., 1987, Green, et al., 1992]. The Tax *trans*-regulator represses *NF1* gene expression through a *cis*-acting element upstream of its transcriptional start site [Feigenbaum, et al., 1996]. Although repression by *tax* occurs in the absence of mutation at the *NF1* locus, transcriptional regulation of the normal or mutant alleles may affect disease pathogenesis in NF1 patients. $Nf1^{+/-}$ mice do not develop the any of the lesions characteristic of human NF1, but mice with inactivating mutations of both *Nf1* and *Trp53* on the same chromosome frequently develop astrocytomas, suggesting that there is biochemical interaction between the products of these two loci. Interestingly, the frequency of astrocytomas in $Nf1^{+/-}$ $Trp53^{+/-}$ cis double heterozygotes varies depending on genetic background [Reilly, et al., 2000]. These models provide evidence that genetic factors at other loci can affect the phenotype associated with *Nf1* mutations but are limited to only a few of NF1 disease features observed in humans.

Easton et al. [Easton, et al., 1993] studied the expressivity of NF1 in 175 affected members of 48 families and found statistically significant correlations for the number of café-au-lait spots, the number of dermal discrete neurofibromas and head circumference among affected relatives. Comparison of the strength of these correlations in relatives of different classes provides

evidence for modifying genes influencing the number of café-au-lait spots. These results rely heavily on near-perfect concordance among 6 pairs of monozygotic twins and were not adjusted for the non-independence of multiple relative-pairs from the same family.

We have shown previously that several statistically significant associations exist between the occurrence of individual clinical features in 3067 unrelated probands with NF1 [Szudek, et al., 2000, Szudek, et al., 1998]. We also found significant associations in the occurrence of Lisch nodules, optic glioma, learning disability, macrocephaly and short stature in affected parent-child pairs [Szudek, et al., 2000], but made no attempt to adjust for the non-independence of multiple relative-pairs from the same family or for associations among clinical features in individuals in this preliminary study. We now extend our analysis to measure correlations of NF1 features among affected sibs, children and their mothers and fathers, and 2nd degree relatives using methods that take other clinical features and age into account and adjust for the non-independence of affected relatives. By comparing the correlations for relatives of various types, we provide evidence that genetic sources of variable expressivity are generally important in NF1 and vary for different clinical features.

SUBJECTS AND METHODS

Subjects

All patients in this study met the NIH diagnostic criteria for NF1 [NIH, 1988, Gutmann, et al., 1997]. Data were obtained from the National NF Foundation International Database (NFDB) [Friedman, et al., 1993] on 913 individuals from 373 families with 2 or more affected members, including 268 sib-sib, 373 parent-child and 74 2^{nd} degree relative pairs.

For analysis of familiality, we selected 12 clinical features of NF1: café-au-lait spots, intertriginous freckling, Lisch nodules, cutaneous neurofibromas, subcutaneous neurofibromas, plexiform neurofibromas, head circumference, stature, seizures, scoliosis, optic glioma and neoplasms other than neurofibromas and optic gliomas ("other neoplasms"). Most of the features were identified by physical examination, and treated as binary variables. Café-au-lait spots were coded as "present" if the subject had 6 or more spots. Cutaneous or subcutaneous neurofibromas were coded as "present" if the subject had two or more lesions of the same type. Plexiform neurofibroma was coded as "present" if the subject had one or more lesions. Stature and head circumference were treated as continuous variables and standardised using population norms [Szudek, et al., 2000]. Lisch nodules were diagnosed or excluded by a slit lamp examination. The presence or absence of optic glioma was determined by cranial MRI or CT examination. Only patients with definite presence or absence of a feature were considered in models involving that feature. The complete data set used in this study is available from the authors by request.

Statistical Methods

We included age as a covariate in all analyses. Age is one of the most important factors influencing the NF1 phenotype [Zöller, et al., 1995]. Many NF1 features, including Lisch nodules, subcutaneous neurofibromas, cutaneous neurofibromas, other neoplasms, intertriginous freckling, seizures and scoliosis have a higher prevalence in older patients [DeBella, et al., 2000]. We have shown previously that clinical features do not occur independently in NF1 patients, even after adjusting for the effect of age [Szudek, et al., 2000, Szudek, et al., 1998]. Therefore, we also controlled for the presence or absence of other associated features to minimize confounding in the present study.

Our general approach was to treat the features of NF1 as if each were a disorder occurring in a population affected with NF1. Familial aggregation of each binary feature among various classes of relatives was estimated using multivariate probit regression models, which assume that each of the binary dependent features reflects an underlying latent quantitative variable. Familial aggregation of continuous features (head circumference and stature) among various classes of relatives was estimated using multivariate normal regression models. The program MPROBIT was used for binary features and MVNFAM for continuous features. Two separate regressions were simultaneously applied to the feature being modelled [Joe, 1997, Joe, 2000]. One regression accounted for covariates such as related features, interactions between related features (each represented by a distinct variable equal to the product of the two interacting features), age and gender. The second regression was used to measure latent correlation of the binary response variable between specific classes of relatives. Alternatively, the second regression was used to measure correlation of the continuous response variable (head circumference or stature) between specific classes of relatives. MPROBIT and MVNFAM provide maximum likelihood estimates

of the regression coefficients and standard errors for each covariate. The programmes also estimate the correlation coefficients and standard errors for the intra-familial relationships specified.

We used the results of a previous study of probands with NF1 from the NFDB [Szudek, et al., 1998] to obtain appropriate functions for age (e.g. $e^{-age/4}$) and initial regression parameter estimates for covariates representing related features, interactions between related features and gender. Familial aggregation was assessed among sibs, parent-child pairs (including mother-child and father-child pairs separately) and 2nd degree relatives. Parameters and coefficients with 95% confidence intervals that excluded zero were deemed statistically significant. Standard errors and covariance matrices were used to test for differences between intra-familial correlation coefficients for different comparisons. For example, to test for a difference between sib-sib correlation and parent-child correlation we used the following formulas:

$$Z = \frac{r_{ss} - r_{pc}}{s} \quad \text{where} \quad s = \sqrt{(SE_{r_{ss}})^2 + (SE_{r_{pc}})^2 - 2\,\mathrm{cov}(r_{ss}, r_{pc})}$$

Z-scores were converted into p-values according to the standard normal distribution. We used one-tailed tests to compare correlations between 1st degree and 2nd degree relatives and between sib pairs and parent-child pairs because we had a prior expectation that correlations between sibs would be at least as strong as those between parents and children [Easton, et al., 1993, Szudek, et al., 2000]. We used two-tailed tests to compare mother-child correlations to father-child correlations.

RESULTS

We studied 913 individuals with NF1 from 373 families with two or more affected members. 91% of the individuals studied were White, 2% Asian, 1% Black, 1% Latin, and the remainder either of "other" or "unknown" origin. Table 1 shows the prevalences of each of the 12 NF1 clinical features in affected fathers, mothers and their affected children in the NFDB study sample and compares them to the prevalences in the sample used by Easton et al. [Easton, et al., 1993].

Familial aggregation among various classes of relatives was estimated using multivariate regression models. Table 2 shows the regression parameters and standard errors for the terms that were included in each model. The strength of association between the modelled feature and a covariate is measured by β. A unit increase in the value of the covariate means the modelled feature is $\exp(2\beta)$ times more likely to be present. For example, subjects with intertriginous freckling were $\exp(2\times.51)=2.8$ times more likely also to have café-au-lait spots than subjects of the same age and gender without intertriginous freckling. Also, subjects with intertriginous freckling *and* subcutaneous neurofibromas were $\exp(2\times(.51-.41+.61))=4.1$ times more likely to also have café-au-lait spots.

The parameter estimates for age were highly significant ($p<0.001$) for Lisch nodules, subcutaneous neurofibromas, cutaneous neurofibromas, and intertriginous freckling; significant ($p<0.05$) for café-au-lait spots, head circumference, stature, optic gliomas and plexiform neurofibromas; and not significant ($p>0.05$) for other neoplasms, seizures and scoliosis. The parameter estimate for gender was not significant in any of the models. However, parameters

that are not statistically significant on their own can still contribute to model interpretation and significance when other related features are also considered.

Table 3 shows the number of sib, parent-child (including mother-child and father child) and 2nd degree relative pairs used in each model. Subjects were included in a model only if the status ("presence" or "absence") of the modelled feature and all covariates was known.

Figure 1 shows the adjusted intrafamilial latent correlation coefficients and their 95% confidence intervals for each of the 12 features among all 913 relatives with NF1 from the 373 families studied. In these estimates, all relatives are treated the same regardless of relationship. Statistically significant positive intrafamilial correlations were observed for Lisch nodules, head circumference, subcutaneous neurofibromas, cutaneous neurofibromas, stature, café-au-lait spots and intertriginous freckling. Correlations for optic glioma, other neoplasms, seizures, scoliosis and plexiform neurofibromas, although positive, were not statistically different from zero. However, the number of individuals who had the latter features, especially optic glioma, other neoplasms, or seizures, was small, and the confidence intervals are very wide.

Figure 2 shows the adjusted intrafamilial correlation coefficients and 95% confidence intervals for 8 clinical features among 746 affected 1st degree relatives and among 148 affected 2nd degree relatives. MPROBIT failed to converge on correlation coefficients between 2nd degree relatives for optic glioma, other neoplasms, seizures or scoliosis because of the low frequency of these features and insufficient sample size. We did obtain correlation coefficients between 1st degree relatives for these features, but none was significantly different from zero. Statistically significant positive correlations between 1st degree relatives were found for 7 of the 8 other features listed in Figure 1. Significant positive correlations between 2nd degree relatives were also found for 4 of these 8 features. Significant negative correlations were not observed for

any of the features. Correlations were significantly greater among 1st degree relatives than among 2nd degree relatives for Lisch nodules (p=0.0001) and café-au-lait spots (p=0.0004). Correlations among 1st degree relatives were not statistically different from correlations among 2nd degree relatives for head circumference (p=0.15), subcutaneous neurofibromas (p=0.06), cutaneous neurofibromas (p=0.49), stature (p=0.30), intertriginous freckling (p=0.07) or plexiform neurofibromas (p=0.11).

Figure 3 shows the adjusted intrafamilial correlation coefficients and 95% confidence intervals for 8 features among 268 affected sib pairs and among 373 affected parent-child pairs. Again, MPROBIT failed to converge on correlation coefficients between sibs or parent-child pairs for optic glioma, other neoplasms, seizures or scoliosis. Statistically significant positive correlations between sibs were found for all 8 features in Figure 3. Significant positive correlations between parents and children were found for 6 of the 8 features. Significant negative correlations were not observed for any of the features. Correlations were significantly greater between sibs than between parents and children for subcutaneous neurofibromas (p=0.04), café-au-lait spots (p=0.001), intertriginous freckling (p=0.03) and plexiform neurofibromas (p=0.02). Correlations between sibs were not statistically different from the correlations between parents and children for Lisch nodules (p=0.40), head circumference (p=0.45), cutaneous neurofibromas (p=0.29), or stature (p=0.20).

Figure 4 shows the adjusted intrafamilial correlation coefficients and 95% confidence intervals for 8 features between 233 affected mother-child pairs and between 140 affected father-child pairs. Statistically significant positive correlations between mothers and children were found for 5 of the 8 features. Significant positive correlations between fathers and children were found for 6 of the 8 features. Significant negative correlations were not observed for any of the

features in either relationship. Correlations between fathers and children are significantly greater than correlations between mothers and children for Lisch nodules ($p=0.001$), subcutaneous neurofibromas ($p=0.0001$) and cutaneous neurofibromas ($p=0.02$). Correlations do not differ significantly between father-child pairs and mother-child pairs for head circumference ($p=0.85$), stature ($p=0.40$), café-au-lait spots ($p=0.62$), intertriginous freckling ($p=0.71$) and plexiform neurofibromas ($p=0.17$).

DISCUSSION

Variable expressivity is a characteristic of many dominantly-inherited human genetic diseases and may have genetic or non-genetic causes. Possible genetic causes of variable expressivity include the effects of differences in the mutant allele, effects of the normal allele, and the effects of modifying genes. For example, analysis of phenotypic variation in von-Hippel-Lindau (VHL) disease has implicated unlinked modifying genes in the pathogenesis of ocular tumours [Webster, et al., 1998], and the risk of ovarian cancer in *BRCA1* mutation carriers is modified by allelic variation at the unlinked *H-RAS* locus [Phelan, et al., 1996]. Our study was designed to evaluate the relative importance of various genetic mechanisms in the interfamilial and intrafamilial variability of NF1.

We analysed familial latent correlations for 10 NF1 clinical features and correlations for 2 NF1 clinical features, while adjusting for other related features, age and gender through statistical modelling. We found 7 of the features to have significant overall intra-familial correlations (Figure 1). We were also able to test for differences between correlations among various classes of relatives for 8 of the 12 features studied. Differences between various classes of relatives were found for 6 of the 7 features with significant overall intra-familial correlations (Figures 2-4).

Several features had significantly positive correlations among 2nd degree relatives, but none were significantly greater than the correlations for the same feature among 1st degree relatives (Figure 2). Similarly, several features had significantly positive correlations between parents and children, but none were greater than correlations for the same feature between sibs (Figure 3). The absence of significant negative correlations supports the statistical validity of our approach.

One would expect to observe negative, as well as positive, correlations by chance when making multiple comparisons.

The NFDB draws its information from specialised clinics, so we were concerned about the representativeness of our sample. Furthermore, patients with unknown status of a feature were excluded from models involving that feature. Nevertheless, frequencies of features found among the familial cases used in this study (Table 1) are comparable to those found in another family study of variable NF1 expressivity [Easton, et al., 1993]. They are also comparable to the feature frequencies from two available population-based studies of NF1 patients [Samuelsson and Axelsson, 1981, Huson, et al., 1989].

Easton et al. [Easton, et al., 1993] studied 175 individuals with NF1 from 48 families, including 6 pairs of monozygotic twins, 76 pairs of sibs, 60 parent-offspring pairs, 54 2^{nd} degree relative pairs and 43 3^{rd} degree relative pairs. They examined 8 NF1 clinical features and found significant intrafamilial correlations for 3 quantitative variables: number of café-au-lait spots, number of cutaneous neurofibromas and head circumference. They also analysed 5 traits as binary variables, but these comparisons did not include adjustments for age. Furthermore, none of their analyses adjusted for the non-independence of multiple relative-pairs from the same family or of various clinical features.

Our sample size is 5 times larger, and we examined 12 clinical features, 6 of which are the same as Easton's. Also, we included associations between features as covariates in the familial analyses. Unlike Easton et al., we did not have counts of café-au-lait spots and dermal discrete neurofibromas, but Easton's quantitative investigations of these features complement our binary analyses nicely. Both studies found evidence of modifying genes on café-au-lait spots, but not on dermal discrete neurofibromas. In all, 10 of our 12 features were treated as binary variables –

we had quantitative data only on stature and head circumference. Many of the clinical features of NF1 (and other diseases) are by nature binary, and ours is the first study to examine correlations for binary traits among different familial relationships while accounting for continuous covariates such as age. Similar methods have been used to study lens opacities [1994] and liver cancer [Liang and Beaty, 1991] in individuals who do not have NF1, but we may be the first to study an autosomal dominant disease in this manner.

Although this is by far the largest group of NF1 families ever studied, we only had 74 pairs of 2^{nd} degree relatives. Models for most features used even fewer 2^{nd} degree relatives because the data were incomplete. Subjects were included in a model only if the status of the modelled feature and all covariates was known (Table 3). These relatively small sample sizes are reflected in the wide 95% confidence intervals for the correlation coefficients among 2^{nd} degree relatives (Figure 2). Furthermore, statistical techniques are less reliable for smaller sample sizes, so we must attach an additional note of caution to the point estimates for the correlation coefficients between 2^{nd} degree relatives, particularly for Lisch nodules, head circumference, stature and intertriginous freckling, in which the analysis included 35 or fewer pairs of 2^{nd} degree relatives (Table 3).

The most important confounding factor in familial analyses of NF1 is age. Many disease features are more prevalent in older NF1 patients [Cnossen, et al., 1998], and, if not appropriately controlled, age might produce a correlation between affected relatives of similar age (e.g., sibs) or obscure a correlation between relatives of very different ages (e.g., parents and children). Our multivariate models minimise the confounding effect of age, but they may not eliminate it completely. The covariate representing age was significant in models for most features, but it is possible that a residual age effect is contributing to the observed differences

between sib and parent-child pairs for features such as subcutaneous neurofibromas and intertriginous freckling that become more prevalent with age (Figure 3). Age is less likely to influence the intrafamilial correlations for café-au-lait spots or plexiform neurofibromas, which, when considered as discrete variables, occur with a relatively stable frequency with age [Riccardi, 1992, DeBella, et al., 2000].

We used one-tailed tests for 1st degree vs. 2nd degree and sib-sib vs. parent-child comparisons. Several of the results just reach a level of nominal statistical significance using one-tailed z-tests, and several others fall only a little short of doing so. Clearly these results require independent confirmation in future studies.

Lisch nodules and café-au-lait spots had significantly higher correlations among 1st degree relatives than among 2nd degree relatives. Higher correlations for 1st than 2nd degree relatives would be expected for effects produced by modifying genes at unlinked loci but might also result from environmental factors that are more likely to be shared among closer relatives. However, it is hard to imagine what environmental factors could contribute to the development of Lisch nodules. No family studies have previously been done on Lisch nodules, and factors contributing to their development are unknown. Our observations are consistent with the effect of a modifying gene on the pathogenesis of Lisch nodules.

Easton et al. [Easton, et al., 1993] found a higher correlation for café-au-lait spots between MZ twins than between sibs, suggesting the effect of a genetic locus or loci in addition to *NF1*. Our findings of a strong correlation for café-au-lait spots in 1st degree relatives but no correlation among 2nd degree relatives are consistent with this interpretation.

Lisch nodules are melanocytic hamartomas that arise in iris tissue [Perry and Font, 1982]. Café-au-lait spots are pigmented macules composed of melanocytes with abnormally large

pigment particles [Fitzpatrick, 1981]. Lisch nodules and café-au-lait spots share an origin from neural crest-derived tissue, but this is also true of some other lesions characteristic of NF1, including neurofibromas of all types and intertriginous freckling [Bolande, 1981]. We previously reported an association between the occurrence of Lisch nodules and café-au-lait spots in individual NF1 patients [Szudek, et al., 2000], but intertriginous freckling was also associated – a feature that shows no indication of a stronger familial correlation among 1st degree than 2nd degree relatives (Figure 2). If the development of Lisch nodules and café-au-lait spots is influenced by modifying genes, it is unclear what the nature of these modifying factors is or whether they are the same or different for these two features.

Intertriginous freckling, subcutaneous neurofibromas, plexiform neurofibromas and café-au-lait spots had higher correlations between sibs than between parents and children. Both sib pairs and parent-child pairs are 1st degree relatives who would be expected to share a similar proportion of non-allelic modifying genes, so the differences we observed in these correlations are unlikely to result from effects of modifying genes. Nevertheless, Easton et al. [Easton, et al., 1993] found that concordance for dermal discrete neurofibromas (which include subcutaneous neurofibromas) between monozygotic twins was much higher than between sibs, an observation that suggests the involvement of a genetic factor. Affected sibs would be expected to share the same normal *NF1* allele by descent half of the time, but parent-child pairs rarely would. Effects of functional polymorphisms of the normal *NF1* allele might explain a higher correlation of these features among sib pairs than among parent-child pairs, but no direct evidence is available on this possibility, and the frequency of functional polymorphisms of the *NF1* locus is unknown. Another possible explanation is differences in environmental factors that are more likely to be shared among sibs than between a parent and child.

Intertriginous freckling, subcutaneous neurofibromas, plexiform neurofibromas and café-au-lait spots all share an origin from neural crest-derived cells. We found that café-au-lait spots and intertriginous freckling tended to occur together in individual NF1 patients, and so did cutaneous, subcutaneous, and plexiform neurofibromas, but associations were not seen between the features in these two groups [Szudek, et al., 1998]. In the present study, we did not find a stronger correlation for cutaneous neurofibromas in sibs than in parent-child pairs, as we did for subcutaneous and plexiform neurofibromas (Figure 3). Intertriginous freckling occurs in skin folds, and local environmental factors may play a role in the development of such freckling [Riccardi, 1992]. There is anecdotal evidence that subcutaneous neurofibromas may also develop as a result of trauma [Riccardi, 1990]. This hypothesis has not been tested formally, and it seems unlikely to account for the development of congenital diffuse plexiform neurofibromas. In any case, it is unclear why factors like cutaneous trauma would be more similar in sibs than in parent-child pairs.

Lisch nodules, subcutaneous neurofibromas, and cutaneous neurofibromas had higher correlations between affected fathers and children than between affected mothers and children (Figure 4). Our sample included twice as many mother-child pairs as father-child pairs, so we were concerned about ascertainment bias – the possibility that only severely affected father-child pairs tend to be seen in the NF clinics that contributed data to the NNFF International Database. However, the frequencies of all features studied were similar in affected fathers as in affected mothers (Table 1).

Shared environment is unlikely to be the sole cause of associations between parents and children, due to large differences in age. It is also unlikely that shared environment is responsible for the difference in correlations between mother-child and father-child pairs.

Likewise, a multifactorial influence with a more extreme threshold for males than for females cannot explain the observations for these features. Gender is not a significant predictive factor in any of our models (Table 2), and feature frequencies among affected children of affected fathers are similar to those among affected children of affected mothers (Table 1). Parent-of-origin effects on severity of NF1 have been suggested [Miller and Hall, 1978, Hall, 1981], but most studies do not support this possibility [Huson, et al., 1989, Riccardi and Wald, 1987]. One study found a male predominance among NF1 patients with pseudarthrosis but no significant parent-of-origin effect [Stevenson, et al., 1999]. Our findings are consistent with a parent-of-origin effect on the strength of the parent-child correlation rather than with a more severe phenotype in affected offspring of parents of one gender when compared to affected offspring of parents of the other gender. Similar parent-child aggregation patterns have been reported for body mass index [Friedlander, et al., 1988] and blood pressure [Hurwich, et al., 1982], but they are unprecedented in NF1. Male-to-male inheritance is unlikely since gender is not a significant factor in any of our models (Table 2) and father-son concordance for Lisch nodules, subcutaneous neurofibromas, cutaneous neurofibromas is the same as father-daughter concordance, which argues against a Y-linked factor. We do not know of a genetic mechanism that can explain this phenomenon in NF1 or for body mass index or blood pressure.

Head circumference and stature had similar correlations for all relationships. This suggests that the mutant *NF1* allele itself is most important in determining these correlations. Easton et al. [Easton, et al., 1993] also found evidence of the importance of the mutant allele in head circumference. The distributions of head circumference and stature in NF1 patients are unimodal [Szudek, et al., 2000], but both NF1 distributions are shifted relative to unaffected norms, suggesting that head circumference and stature are affected to a degree in all NF1 patients.

Taken together with the results of the present study, it appears that the magnitude of this effect depends, at least partly, on the mutant *NF1* allele. The mutant *NF1* genotype also has a very strong effect on the phenotypic manifestations in patients with Watson syndrome [Allanson, et al., 1991] or deletions of the whole *NF1* locus [Tonsgard, et al., 1997, Dorschner, et al., 2000].

The patterns of familial correlations shown here suggest that genetic factors involved in determining the occurrence of various clinical features of NF1 vary depending on the feature. In some instances, the mutant *NF1* allele may be most important. In other instances, the effects of the normal *NF1* allele or of unlinked modifying genes may predominate. More than one genetic factor may be involved, and the relative importance of various genetic and non-genetic effects may vary for different features.

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REFERENCES

Abeliovich D, Gelman-Kohan Z, Silverstein S, Lerer I, Chemke J, Merlin S, Zlotogora J (1995) Familial café au lait spots: a variant of neurofibromatosis type 1. J Med Genet 32:985-986

Allanson J, Upadhaya M, Watson G, Partington M, MacKenzie A, Lahey D, MacLeod H, Sarfarazi M, Broadhead W, Harper P, Huson S (1991) Watson syndrome: Is it a subtype of type 1 neurofibromatosis? J Med Genet 28:752-756

Ars E, Kruyer H, Gaona A, Casquero P, Rosell J, Volpini V, Serra E, Lázaro C, Estavill X (1998) A clinical variant of neurofibromatosis type 1: Familial spinal neurofibromatosis with a frameshift mutation in the NF1 gene. Am J Hum Genet 62:834-841

Bolande R (1981) Neurofibromatosis - the quintessential neurocristopathy: Pathogenic concepts and relationships. Adv Neurol 29:67-75

Cnossen M, de Goede-Bolder A, van den Broek K, Waasdorp C, Oranje A, Stroink H, Simonsz H, van den Ouweland A, Halley D, Niermeijer M (1998) A prospective 10 year follow up study of patients with neurofibromatosis type 1. Arch Dis Child 78:408-412

DeBella K, Szudek J, Friedman JM (2000) Use of the national institutes of health criteria for diagnosis of neurofibromatosis 1 in children. Pediatrics 105:608-14

Dorschner MO, Sybert VP, Weaver M, Pletcher BA, Stephens K (2000) NF1 microdeletion breakpoints are clustered at flanking repetitive sequences. Hum Mol Genet 9:35-46

Easton D, Ponder M, Huson S, Ponder B (1993) An analysis of variation in expression of neurofibromatosis (NF) type I (NFI): Evidence for modifying genes. Am J Hum Genet 53:305-313

Fahsold R, Hoffmeyer S, Mischung C, Gille C, Ehlers C, Kucukceylan N, Abdel-Nour M, Gewies A, Peters H, Kaufmann D, Buske A, Tinschert S, Nurnberg P (2000) Minor lesion mutational spectrum of the entire NF1 gene does not explain its high mutability but points to a functional domain upstream of the GAP-related domain. Am J Hum Genet 66:790-818

Feigenbaum L, Fujita K, Collins FS, Jay G (1996) Repression of the NF1 gene by Tax may expain the development of neurofibromas in human T-lymphotropic virus type 1 transgenic mice. J Virol 70:3280-5

Fitzpatrick TB (1981) Melanin synthesis pathways in the pathogenesis of neurofibromatosis. Adv Neurol 29:209-11

Framingham Eye Study (1994) Familial aggregation of lens opacities: the Framingham Eye Study and the Framingham Offspring Eye Study. Am J Epidemiol 140:555-64

Friedlander Y, Kark JD, Kaufmann NA, Berry EM, Stein Y (1988) Familial aggregation of body mass index in ethnically diverse families in Jerusalem. The Jerusalem Lipid Research Clinic. Int J Obes 12:237-47

Friedman J, Greene C, Birch P, and the NNFF International Database P (1993) National Neurofibromatosis Foundation International Database. Am J Med Genet 45:88-91

Friedman J, Riccardi V (1999) Clinical and epidemiological features. In: Friedman J, Gutmann D, MacCollin M, Riccardi V (eds) Neurofibromatosis : phenotype, natural history, and pathogenesis. Johns Hopkins University Press, Baltimore, pp 29-86

Friedman JM (1999) Epidemiology of neurofibromatosis type 1. Am J Med Genet 89:1-6

Green JE, Baird AM, Hinrichs SH, Klintworth GK, Jay G (1992) Adrenal medullary tumors and iris proliferation in a transgenic mouse model of neurofibromatosis. Am J Pathol 140:1401-10

Gutmann D, Aylsworth A, Carey J, Korf B, Marks J, Pyeritz R, Rubenstein A, Viskochil D (1997) The diagnostic evaluation and multidisciplinary management of neurofibromatosis 1 and neurofibromatosis 2. JAMA 278:51-57

Hall J (1981) Possible maternal and hormonal factors in neurofibromatosis. Adv Neurol 29:125-131

Heim RA, Kam-Morgan LN, Binnie CG, Corns DD, Cayouette MC, Farber RA, Aylsworth AS, Silverman LM, Luce MC (1995) Distribution of 13 truncating mutations in the neurofibromatosis 1 gene. Hum Mol Genet 4:975-81

Hinrichs SH, Nerenberg M, Reynolds RK, Khoury G, Jay G (1987) A transgenic mouse model for human neurofibromatosis. Science 237:1340-3

Hurwich BJ, Rosner B, Nubani N, Kass EH, Lewitter FI (1982) Familial aggregation of blood pressure in a highly inbred community, Abu Ghosh, Israel. Am J Epidemiol 115:646-56

Huson S, Compston D, Clark P, Harper P (1989) A genetic study of von Recklinghausen neurofibromatosis in south east Wales: I. Prevalence, fitness, mutation rate, and effect of parental transmission on severity. J Med Genet 26:704-711

Joe H (1997) Multivariate models and dependence concepts Monographs on statistics and applied probability ; 73. Chapman & Hall, London ; New York, pp xviii, 399

Joe H (2000) Programs for multivariate binary (logit/probit) models

Korf B (1999) NNFF International NF1 Genetic Analysis Consortium Mutation Summary Data. National Neurofibromatosis Foundation

Li Y, O'Connell P, Breidenbach HH, Cawthon R, Stevens J, Xu G, Neil S, Robertson M, White R, Viskochil D (1995) Genomic organization of the neurofibromatosis 1 gene (NF1). Genomics 25:9-18

Liang KY, Beaty TH (1991) Measuring familial aggregation by using odds-ratio regression models. Genet Epidemiol 8:361-70

Messiaen LM, Callens T, Mortier G, Beysen D, Vandenbroucke I, Van Roy N, Speleman F, Paepe AD (2000) Exhaustive mutation analysis of the NF1 gene allows identification of 95% of mutations and reveals a high frequency of unusual splicing defects. Hum Mutat 15:541-55

Miller M, Hall J (1978) Possible maternal effect on severity of neurofibromatosis. Lancet 2:1071-1073

Nerenberg M, Hinrichs SH, Reynolds RK, Khoury G, Jay G (1987) The tat gene of human T-lymphotropic virus type 1 induces mesenchymal tumors in transgenic mice. Science 237:1324-9

NIH (1988) Neurofibromatosis: Conference statement. National Institutes of Health Consensus Development Conference. Arch Neurol 45:575-8

Park VM, Pivnick EK (1998) Neurofibromatosis type 1 (NF1): a protein truncation assay yielding identification of mutations in 73% of patients. J Med Genet 35:813-20

Perry H, Font R (1982) Iris nodules in von Recklinghausen's neurofibromatosis: Electron microscopic confirmation of their melanocytic origin. Arch Ophthalmol 100:1635-1640

Phelan CM, Rebbeck TR, Weber BL, Devilee P, Ruttledge MH, Lynch HT, Lenoir GM, Stratton MR, Easton DF, Ponder BA, Cannon-Albright L, Larsson C, Goldgar DE, Narod SA

(1996) Ovarian cancer risk in BRCA1 carriers is modified by the HRAS1 variable number of tandem repeat (VNTR) locus. Nat Genet 12:309-11

Poyhonen M, Leisti E-L, Kytölä S, Leisti J (1997) Hereditary spinal neurofibromatosis: A rare form of NF1? J Med Genet 34:184-187

Pulst S, Riccardi V, Fain P, Korenberg J (1991) Familial spinal neurofibromatosis: Clinical and DNA linkage analysis. Neurology 41:1923-1927

Reilly KM, Loisel DA, Bronson RT, McLaughlin ME, Jacks T (2000) Nf1;Trp53 mutant mice develop glioblastoma with evidence of strain-specific effects. Nat Genet 26:109-13

Riccardi V (1990) The potential role of trauma and mast cells in the pathogenesis of neurofibromas. In: Ishibashi Y, Hori Y (eds) Tuberous sclerosis and neurofibromatosis : epidemiology, pathophysiology, biology, and management. Elsevier, Amsterdam, pp 167-190

Riccardi V (1992) Neurofibromatosis: Phenotype, natural history, and pathogenesis. The Johns Hopkins University Press, Baltimore

Riccardi V, Wald J (1987) Discounting an adverse maternal effect on severity of neurofibromatosis. Pediatrics 79:386-393

Samuelsson B, Axelsson R (1981) Neurofibromatosis: A clinical and genetic study of 96 cases in Gothenburg, Sweden. Acta Dermatovenereolog Suppl 95:67-71

Stevenson DA, Birch PH, Friedman JM, Viskochil DH, Balestrazzi P, Boni S, Buske A, Korf BR, Niimura M, Pivnick EK, Schorry EK, Short MP, Tenconi R, Tonsgard JH, Carey JC (1999) Descriptive analysis of tibial pseudarthrosis in patients with neurofibromatosis 1. Am J Med Genet 84:413-9

Szudek J, Birch P, Friedman JM (2000a) Growth in north american white children with neurofibromatosis 1 (NF1). J Med Genet 37:933-8

Szudek J, Birch P, Riccardi VM, Evans DG, Friedman JM (2000b) Associations of clinical features in neurofibromatosis 1 (NF1). Genet Epidemiol 19:429-39

Szudek J, Evans D, Friedman J (1998) Logistic regresssive models of neurofibromatosis 1 (NF1) clinical features American Society of Human Genetics Annual Meeting. Am J Hum Genet, Baltimore

Tonsgard J, Yalavarthi K, Cushner S, Short M, Lindgren V (1997) Do NF1 gene deletions result in a characteristic phenotype? Am J Med Genet 73:80-86

Viskochil D (1999) The structure and function of the NF1 Gene: molecular pathophysiology. In: Friedman J, Gutmann D, MacCollin M, Riccardi V (eds) Neurofibromatosis : phenotype, natural history, and pathogenesis. Johns Hopkins University Press, Baltimore, pp 119-141

Webster AR, Richards FM, MacRonald FE, Moore AT, Maher ER (1998) An analysis of phenotypic variation in the familial cancer syndrome von Hippel-Lindau disease: evidence for modifier effects. Am J Hum Genet 63:1025-35

Zöller M, Rembeck B, Åkesson H, Angervall L (1995) Life expectancy, mortality and prognostic factors in neurofibromatosis type 1: A twelve-year follow-up of an epidemiological study in Göteborg, Sweden. Acta Derm Venerol (Stockh) 75:136-140

Table 1. Number and percentage of subjects from the NFDB and from the study by Easton et al. with various NF1 features. Features not considered by Easton et al. have empty cells in the last two columns.

Feature	NFDB								Easton et al.	
	Affected Fathers		Affected Mothers		Affected Children of Affected Fathers		Affected Children of Affected Mothers		All Affected Relatives	
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
Lisch nodules	63	(81%)	101	(80%)	64	(63%)	101	(51%)	409	(60%)		
Café-au-lait macules	67	(68%)	125	(73%)	115	(79%)	181	(74%)	657	(75%)	129	(88%)
Cutaneous neurofibromas	78	(80%)	119	(69%)	37	(25%)	67	(27%)	355	(40%)	121	(76%)
Optic glioma	4	(13%)	2	(4%)	8	(14%)	14	(15%)	45	(15%)	9	(5%)
Subcutaneous neurofibromas	60	(61%)	99	(59%)	31	(21%)	58	(23%)	291	(33%)		
Intertriginous freckling	79	(83%)	147	(88%)	112	(78%)	191	(78%)	699	(80%)		
Seizures	7	(7%)	12	(7%)	8	(5%)	16	(6%)	58	(6%)	12	(7%)
Plexiform neurofibromas	24	(24%)	35	(20%)	25	(18%)	44	(18%)	176	(20%)	37	(21%)
Scoliosis	7	(8%)	8	(6%)	29	(22%)	32	(14%)	96	(12%)	27	(16%)
Other neoplasms	4	(4%)	11	(6%)	4	(3%)	7	(3%)	33	(4%)		

Table 2. Summary of regressions in multivariate models for 12 clinical NF1 features. The 1st column lists the 12 modelled features. The 2nd–4th columns show the covariates and their regression parameter estimates (β) with standard errors (SE) used in each model. β_0 is the intercept in the model equation. Each regression accounts for covariates such as related features, interactions between related features, age and gender. Interactions are depicted by features separated by an "*" and their values equal the product of the two interacting features.

Modelled Feature	**Intercept and Covariates**	β	**SE**
Lisch nodules	β_0	.65	(.08)
	Age	-3.55	(.32)
	Male gender	-.01	(.08)
	Café-au-lait spots	.23	(.15)
	Cutaneous neurofibromas	.44	(.20)
	Café-au-lait spots * Cutaneous neurofibromas	-.09	(.22)
Café-au-lait spots	β_0	.28	(.14)
	Age	-.66	(.25)
	Male gender	.03	(.09)
	Intertriginous freckling	.51	(.12)
	Subcutaneous neurofibromas	-.41	(.26)
	Intertriginous freckling* Subcutaneous neurofibromas	.61	(.28)
Head circumference	β_0	-.99	(.10)
	Age	.62	(.21)
	Male gender	-.09	(.31)
	Lisch nodules	-.06	(.36)
	Optic glioma	.56	(.44)
	Stature	.34	(.04)
	Neoplasms	.10	(.75)
Cutaneous neurofibromas	β_0	-1.62	(.11)
	Age	-5.56	(.36)
	Male gender	.01	(.10)
	Subcutaneous neurofibromas	.62	(.11)
	Plexiform neurofibromas	.36	(.12)
Stature	β_0	-.62	(.09)
	Age	-.82	(.31)
	Male gender	-.03	(.09)
	Head circumference	.04	(.01)
Optic glioma	β_0	-1.02	(.13)
	Age	.72	(.57)
	Male gender	.06	(.17)
	Plexiform neurofibromas	.01	(.37)
	Head circumference	.19	(.07)
	Neoplasms	.55	(.49)

Table 2 (continued)

Modelled Feature	Intercept and Covariates	β	SE
Subcutaneous neurofibromas	β_0	-1.72	(.12)
	Age	-3.78	(.35)
	Male gender	-.04	(.08)
	Café-au-lait spots	.43	(.11)
	Cutaneous neurofibromas	.73	(.13)
	Plexiform neurofibromas	.52	(.17)
	Intertriginous freckling * Plexiform neurofibromas	-.24	(.23)
Intertriginous freckling	β_0	.49	(.15)
	Age	-1.58	(.30)
	Male gender	-.23	(.12)
	Café-au-lait spots	.52	(.14)
	Subcutaneous neurofibromas	-.18	(.27)
	Lisch nodules	.55	(.14)
	Café-au-lait spots * Subcutaneous neurofibromas	.62	(.33)
Seizures	β_0	-1.43	(.11)
	Age	-.88	(.65)
	Male gender	-.04	(.15)
Plexiform neurofibromas	β_0	-1.11	(.11)
	Age	-.88	(.38)
	Male gender	.07	(.09)
	Subcutaneous neurofibromas	.46	(.16)
	Cutaneous neurofibromas	.37	(.14)
	Subcutaneous * Cutaneous neurofibromas	-.21	(.22)
Scoliosis	β_0	-1.11	(.09)
	Age	-.57	(.34)
	Male gender	-.02	(.11)
Other neoplasms	β_0	-.95	(.23)
	Age	-4.07	(2.11)
	Male gender	-.06	(.21)
	Lisch nodules	-.55	(.25)
	Optic glioma	.32	(.31)

Table 3. Number of relatives used in multivariate probit models for 12 clinical NF1 features. The 1st column lists the 12 modelled features. The 2nd-5th columns show the number of affected sib, mother-child, father-child and 2nd degree relative pairs for which the status of the modelled feature and covariates in Table 2 is known.

Modelled Feature	**Sib Pairs**	**Mother-Child Pairs**	**Father-Child Pairs**	**2° Relative Pairs**
Lisch nodules	192	159	79	35
Café-au-lait macules	248	210	129	69
Head circumference	103	64	37	24
Cutaneous neurofibromas	264	224	131	69
Stature	103	64	37	24
Optic glioma	55	37	26	4
Subcutaneous neurofibromas	253	220	131	69
Intertriginous freckling	179	148	75	35
Seizures	268	233	140	74
Plexiform neurofibromas	264	224	131	69
Scoliosis	228	191	131	53
Other neoplasms	47	33	20	3

Figure 1: Adjusted intrafamilial latent correlation coefficients and their 95% confidence intervals for each of the 12 features among all 913 relatives with NF1 from the 373 families studied. In these estimates, all relatives are treated the same regardless of relationship.

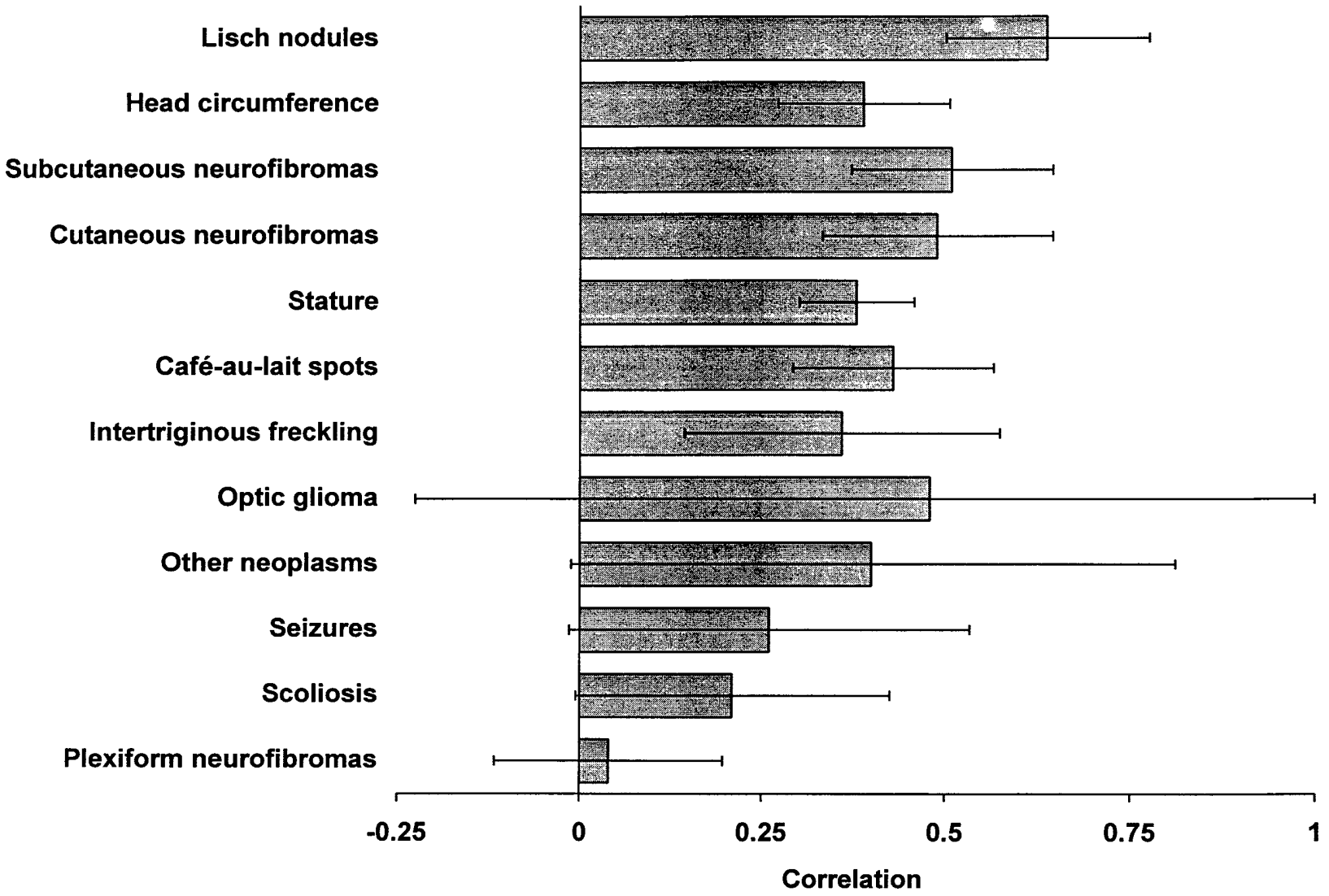

Lisch nodules
Head circumference
Subcutaneous neurofibromas
Cutaneous neurofibromas
Stature
Café-au-lait spots
Intertriginous freckling
Optic glioma
Other neoplasms
Seizures
Scoliosis
Plexiform neurofibromas
-0.25
0
0.25
0.5
0.75
1
Correlation

Figure 2: Adjusted intrafamilial correlation coefficients and 95% confidence intervals for 8 clinical features among 746 affected 1st degree relatives and among 148 affected 2nd degree relatives. A star indicates a significant difference between the correlation coefficients of the two classes being compared. MPROBIT failed to converge on correlation coefficients between 2nd degree relatives for optic glioma, other neoplasms, seizures or scoliosis because of the low frequency of these features and insufficient sample size.

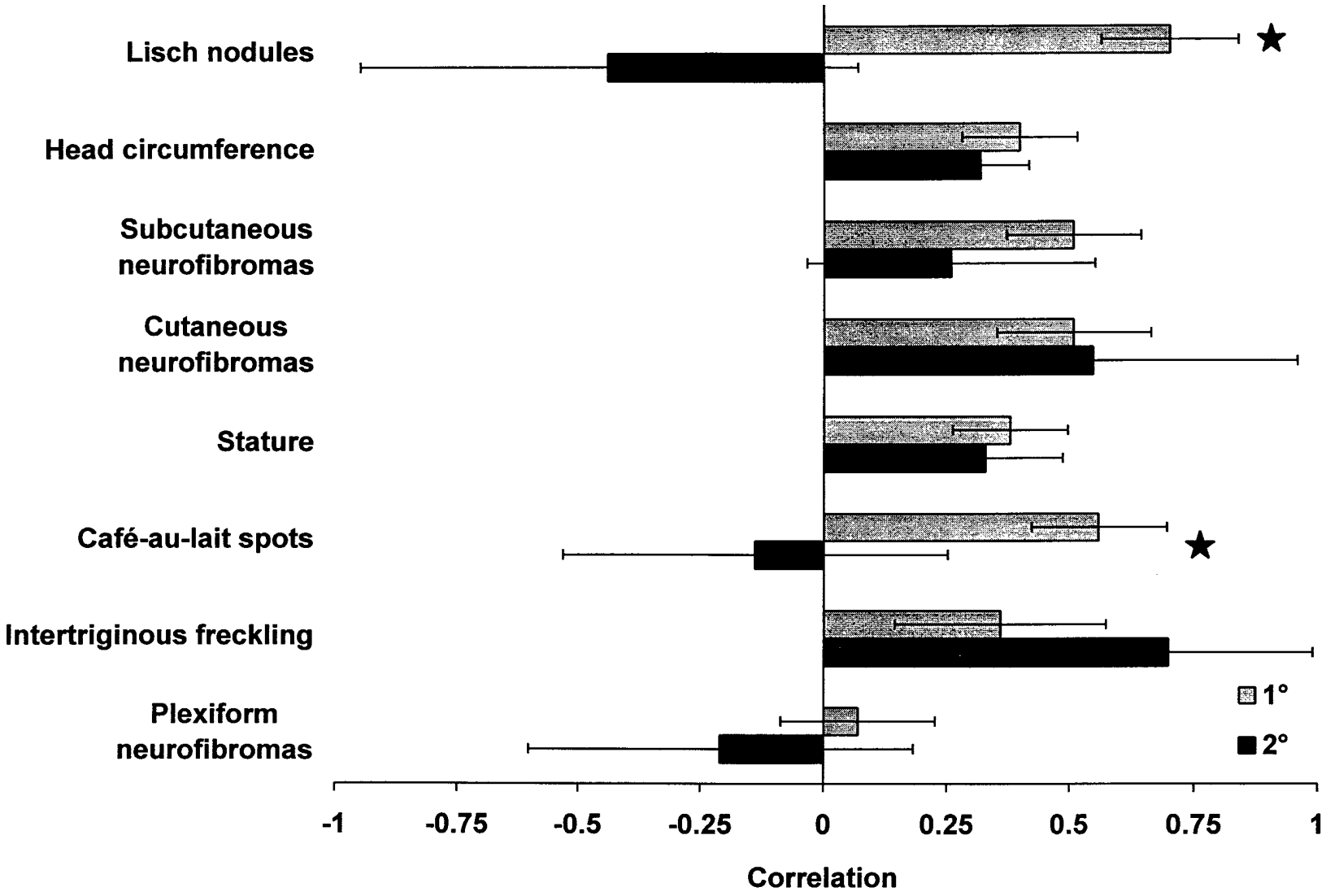

Lisch nodules
Head circumference
Subcutaneous neurofibromas
Cutaneous neurofibromas
Stature
Café-au-lait spots
Intertriginous freckling
Plexiform neurofibromas
1°
2°
-1
-0.75
-0.5
-0.25
0
0.25
0.5
0.75
1
Correlation

Figure 3: Adjusted intrafamilial correlation coefficients and 95% confidence intervals for 8 features among 268 affected sib pairs and among 373 affected parent-child pairs. A star indicates a significant difference between the correlation coefficients of the two classes being compared. MPROBIT failed to converge on correlation coefficients between sibs or parent-child pairs for optic glioma, other neoplasms, seizures or scoliosis.

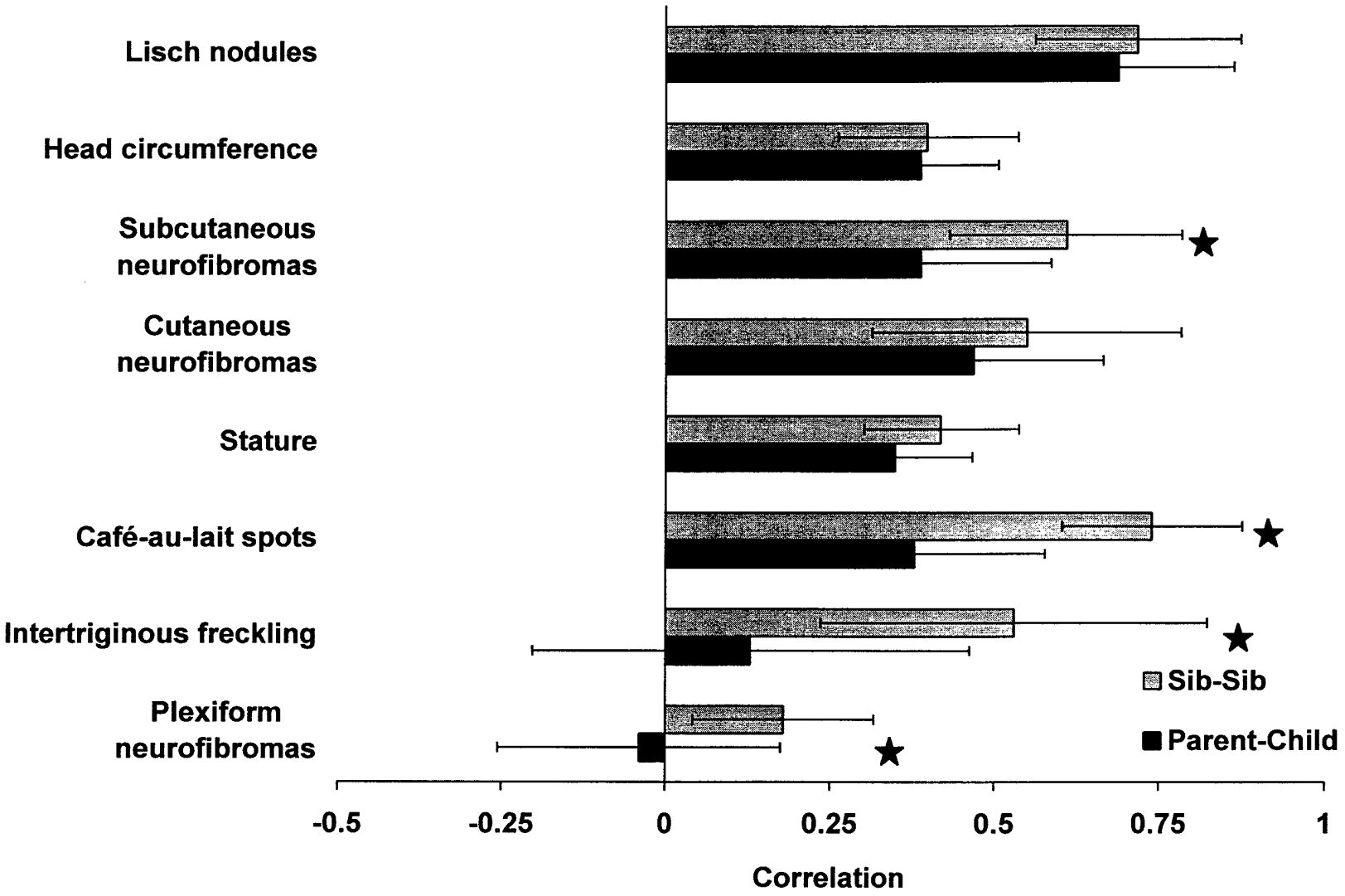
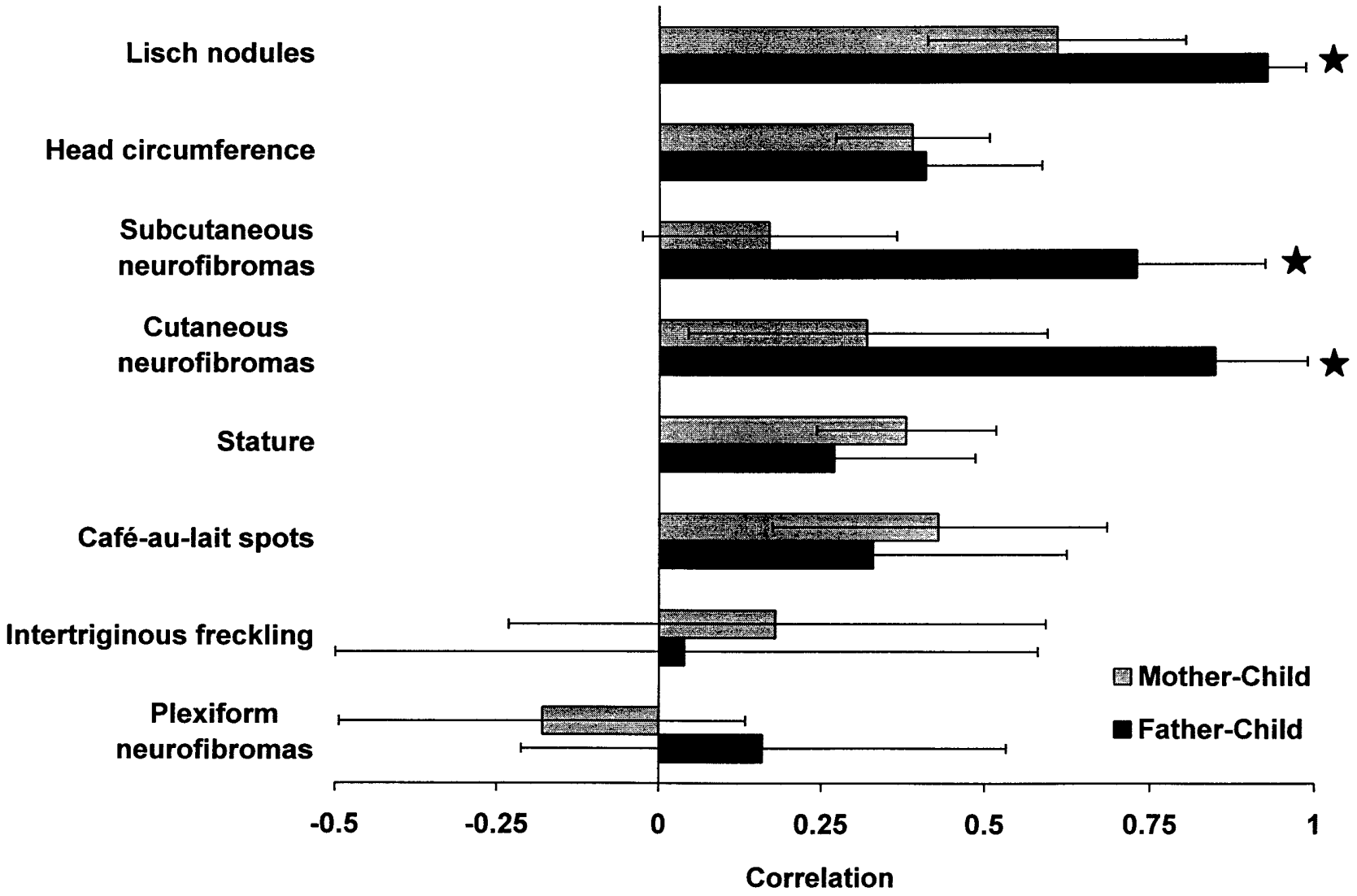

Lisch nodules
Head circumference
Subcutaneous neurofibromas
Cutaneous neurofibromas
Stature
Café-au-lait spots
Intertriginous freckling
Plexiform neurofibromas
Sib-Sib
Parent-Child
-0.5
-0.25
0
0.25
0.5
0.75
1
Correlation

Figure 4: Adjusted intrafamilial correlation coefficients and 95% confidence intervals for 8 features between 233 affected mother-child pairs and between 140 affected father-child pairs. A star indicates a significant difference between the correlation coefficients of the two classes being compared.


Lisch nodules
Head circumference
Subcutaneous neurofibromas
Cutaneous neurofibromas
Stature
Café-au-lait spots
Intertriginous freckling
Plexiform neurofibromas
Mother-Child
Father-Child
-0.5
-0.25
0
0.25
0.5
0.75
1
Correlation

Friedman JM. 1999. Epidemiology of neurofibromatosis type 1. Am J Med Genet 89:1-6.

Riccardi V. 1992. Neurofibromatosis: Phenotype, natural history, and pathogenesis. Baltimore: The Johns Hopkins University Press.

Friedman J and Riccardi V. 1999. Clinical and epidemiological features. In: Friedman J, Gutmann D, MacCollin M and Riccardi V editors. Neurofibromatosis : phenotype, natural history, and pathogenesis. Baltimore: Johns Hopkins University Press. p 29-86.

Li Y, O'Connell P, Breidenbach HH, Cawthon R, Stevens J, Xu G, Neil S, Robertson M, White R and Viskochil D. 1995. Genomic organization of the neurofibromatosis 1 gene (NF1). Genomics 25:9-18.

Viskochil D. 1999. The structure and function of the NF1 Gene: molecular pathophysiology. In: Friedman J, Gutmann D, MacCollin M and Riccardi V editors. Neurofibromatosis : phenotype, natural history, and pathogenesis. Baltimore: Johns Hopkins University Press. p 119-141.

Heim RA, Kam-Morgan LN, Binnie CG, Corns DD, Cayouette MC, Farber RA, Aylsworth AS, Silverman LM and Luce MC. 1995. Distribution of 13 truncating mutations in the neurofibromatosis 1 gene. Hum Mol Genet 4:975-81.

Park VM and Pivnick EK. 1998. Neurofibromatosis type 1 (NF1): a protein truncation assay yielding identification of mutations in 73% of patients. J Med Genet 35:813-20.

Messiaen LM, Callens T, Mortier G, Beysen D, Vandenbroucke I, Van Roy N, Speleman F and Paepe AD. 2000. Exhaustive mutation analysis of the NF1 gene allows identification of 95% of mutations and reveals a high frequency of unusual splicing defects. Hum Mutat 15:541-55.

Korf B. 1999. NNFF International NF1 Genetic Analysis Consortium Mutation Summary Data.

Fahsold R, Hoffmeyer S, Mischung C, Gille C, Ehlers C, Kucukceylan N, Abdel-Nour M, Gewies A, Peters H, Kaufmann D, et al. 2000. Minor lesion mutational spectrum of the entire NF1 gene does not explain its high mutability but points to a functional domain upstream of the GAP-related domain. Am J Hum Genet 66:790-818.

Tonsgard J, Yalavarthi K, Cushner S, Short M and Lindgren V. 1997. Do NF1 gene deletions result in a characteristic phenotype? Am J Med Genet 73:80-86.

Dorschner MO, Sybert VP, Weaver M, Pletcher BA and Stephens K. 2000. NF1 microdeletion breakpoints are clustered at flanking repetitive sequences. Hum Mol Genet 9:35-46.

Allanson J, Upadhaya M, Watson G, Partington M, MacKenzie A, Lahey D, MacLeod H, Sarfarazi M, Broadhead W, Harper P, et al. 1991. Watson syndrome: Is it a subtype of type 1 neurofibromatosis? J Med Genet 28:752-756.

Abeliovich D, Gelman-Kohan Z, Silverstein S, Lerer I, Chemke J, Merlin S and Zlotogora J. 1995. Familial café au lait spots: a variant of neurofibromatosis type 1. J Med Genet 32:985-986.

Pulst S, Riccardi V, Fain P and Korenberg J. 1991. Familial spinal neurofibromatosis: Clinical and DNA linkage analysis. Neurology 41:1923-1927.

Poyhonen M, Leisti E-L, Kytölä S and Leisti J. 1997. Hereditary spinal neurofibromatosis: A rare form of NF1? J Med Genet 34:184-187.

Ars E, Kruyer H, Gaona A, Casquero P, Rosell J, Volpini V, Serra E, Lázaro C and Estavill X. 1998. A clinical variant of neurofibromatosis type 1: Familial spinal neurofibromatosis with a frameshift mutation in the NF1 gene. Am J Hum Genet 62:834-841.

Hinrichs SH, Nerenberg M, Reynolds RK, Khoury G and Jay G. 1987. A transgenic mouse model for human neurofibromatosis. Science 237:1340-3.

Nerenberg M, Hinrichs SH, Reynolds RK, Khoury G and Jay G. 1987. The tat gene of human T-lymphotropic virus type 1 induces mesenchymal tumors in transgenic mice. Science 237:1324-9.

Green JE, Baird AM, Hinrichs SH, Klintworth GK and Jay G. 1992. Adrenal medullary tumors and iris proliferation in a transgenic mouse model of neurofibromatosis. Am J Pathol 140:1401-10.

Feigenbaum L, Fujita K, Collins FS and Jay G. 1996. Repression of the NF1 gene by Tax may expain the development of neurofibromas in human T-lymphotropic virus type 1 transgenic mice. J Virol 70:3280-5.

Reilly KM, Loisel DA, Bronson RT, McLaughlin ME and Jacks T. 2000. Nf1;Trp53 mutant mice develop glioblastoma with evidence of strain-specific effects. Nat Genet 26:109-13.

Easton D, Ponder M, Huson S and Ponder B. 1993. An analysis of variation in expression of neurofibromatosis (NF) type I (NFI): Evidence for modifying genes. Am J Hum Genet 53:305-313.

Szudek J, Birch P, Riccardi VM, Evans DG and Friedman JM. 2000. Associations of clinical features in neurofibromatosis 1 (NF1). Genet Epidemiol 19:429-39.

Szudek J, Evans D and Friedman J. 1998. Logistic regresssive models of neurofibromatosis 1 (NF1) clinical features.

NIH. 1988. Neurofibromatosis: Conference statement. National Institutes of Health Consensus Development Conference. Arch Neurol 45:575-8.

Gutmann D, Aylsworth A, Carey J, Korf B, Marks J, Pyeritz R, Rubenstein A and Viskochil D. 1997. The diagnostic evaluation and multidisciplinary management of neurofibromatosis 1 and neurofibromatosis 2. JAMA 278:51-57.

Friedman J, Greene C, Birch P and and the NNFF International Database P. 1993. National Neurofibromatosis Foundation International Database. Am J Med Genet 45:88-91.

Szudek J, Birch P and Friedman JM. 2000. Growth in North American white children with neurofibromatosis 1 (NF1). J Med Genet 37:933-8.

Zöller M, Rembeck B, Åkesson H and Angervall L. 1995. Life expectancy, mortality and prognostic factors in neurofibromatosis type 1: A twelve-year follow-up of an epidemiological study in Göteborg, Sweden. Acta Derm Venerol (Stockh) 75:136-140.

DeBella K, Szudek J and Friedman JM. 2000. Use of the national institutes of health criteria for diagnosis of neurofibromatosis 1 in children. Pediatrics 105:608-14.

Joe H. 1997. Multivariate models and dependence concepts. London ; New York: Chapman & Hall.

Joe H. 2000. Programs for multivariate binary (logit/probit) models. (Available from ftp://ftp.stat.ubc.ca/pub/hjoe/mbin/).

Webster AR, Richards FM, MacRonald FE, Moore AT and Maher ER. 1998. An analysis of phenotypic variation in the familial cancer syndrome von Hippel-Lindau disease: evidence for modifier effects. Am J Hum Genet 63:1025-35.

Phelan CM, Rebbeck TR, Weber BL, Devilee P, Ruttledge MH, Lynch HT, Lenoir GM, Stratton MR, Easton DF, Ponder BA, et al. 1996. Ovarian cancer risk in BRCA1 carriers is modified by the HRAS1 variable number of tandem repeat (VNTR) locus. Nat Genet 12:309-11.

Samuelsson B and Axelsson R. 1981. Neurofibromatosis: A clinical and genetic study of 96 cases in Gothenburg, Sweden. Acta Dermatovenereolog Suppl 95:67-71.

Huson S, Compston D, Clark P and Harper P. 1989. A genetic study of von Recklinghausen neurofibromatosis in south east Wales: I. Prevalence, fitness, mutation rate, and effect of parental transmission on severity. J Med Genet 26:704-711.

Framingham. 1994. Familial aggregation of lens opacities: the Framingham Eye Study and the Framingham Offspring Eye Study. Am J Epidemiol 140:555-64.

Liang KY and Beaty TH. 1991. Measuring familial aggregation by using odds-ratio regression models. Genet Epidemiol 8:361-70.

Cnossen M, de Goede-Bolder A, van den Broek K, Waasdorp C, Oranje A, Stroink H, Simonsz H, van den Ouweland A, Halley D and Niermeijer M. 1998. A prospective 10 year follow up study of patients with neurofibromatosis type 1. Arch Dis Child 78:408-412.

Perry H and Font R. 1982. Iris nodules in von Recklinghausen's neurofibromatosis: Electron microscopic confirmation of their melanocytic origin. Arch Ophthalmol 100:1635-1640.

Fitzpatrick TB. 1981. Melanin synthesis pathways in the pathogenesis of neurofibromatosis. Adv Neurol 29:209-11.

Bolande R. 1981. Neurofibromatosis - the quintessential neurocristopathy: Pathogenic concepts and relationships. Adv Neurol 29:67-75.

Riccardi V. 1990. The potential role of trauma and mast cells in the pathogenesis of neurofibromas. In: Ishibashi Y and Hori Y editors. Tuberous sclerosis and neurofibromatosis : epidemiology, pathophysiology, biology, and management. Amsterdam: Elsevier. p 167-190.

Miller M and Hall J. 1978. Possible maternal effect on severity of neurofibromatosis. Lancet 2:1071-1073.

Hall J. 1981. Possible maternal and hormonal factors in neurofibromatosis. Adv Neurol 29:125-131.

Riccardi V and Wald J. 1987. Discounting an adverse maternal effect on severity of neurofibromatosis. Pediatrics 79:386-393.

Stevenson DA, Birch PH, Friedman JM, Viskochil DH, Balestrazzi P, Boni S, Buske A, Korf BR, Niimura M, Pivnick EK, et al. 1999. Descriptive analysis of tibial pseudarthrosis in patients with neurofibromatosis 1. Am J Med Genet 84:413-9.

Friedlander Y, Kark JD, Kaufmann NA, Berry EM and Stein Y. 1988. Familial aggregation of body mass index in ethnically diverse families in Jerusalem. The Jerusalem Lipid Research Clinic. Int J Obes 12:237-47.

Hurwich BJ, Rosner B, Nubani N, Kass EH and Lewitter FI. 1982. Familial aggregation of blood pressure in a highly inbred community, Abu Ghosh, Israel. Am J Epidemiol 115:646-56.

Familial aggregation of neurofibromatosis 1 (NF1) clinical features. *J. Szudek[1], H. Joe[2], J.M. Friedman[1].* 1) Departments of Medical Genetics and; 2) Statistics, University of British Columbia, Vancouver, BC, Canada.

The relationship of genetic factors at the *NF1* locus or other loci to development of specific disease features is poorly understood. This study examines familial aggregation of NF1 features among different classes of affected relatives.

The National NF Foundation International Database contains data on 320 families with ≥2 members affected with NF1, including 223 sib-sib, 290 parent-child and 70 second degree relative pairs. For this study, we selected 10 NF1 clinical features: café-au-lait spots, intertriginous freckling, cutaneous, subcutaneous and plexiform neurofibromas, Lisch nodules, seizures, scoliosis, macrocephaly and short stature. The probit of each feature was set as the response in a different model. Two separate regressions were simultaneously applied with each feature as the response. One accounted for related features and covariates such as age and gender. The other measured aggregation of the response variable between affected sibs, parent-child pairs and second degree relatives.

All of the features except seizures and scoliosis appear to be familial. Among the familial features, correlations between sibs ranged from 0.30 (95% CI 0.07-0.54) for plexiform neurofibromas to 0.72 (95% CI 0.52-0.92) for Lisch nodules. Correlations between parents and children ranged from 0.24 (95% CI 0.04-0.44) for subcutaneous neurofibromas to 0.73 (95% CI 0.48-0.98) for Lisch nodules. Correlations between second degree relatives ranged from -0.18 (95% CI -0.40-0.04) for Lisch nodules to 0.59 (95% CI 0.06-1.00) for macrocephaly. Three distinct patterns were observed among the correlations for familial features: 1) Some features had similar correlations for all relationships; 2) Others had higher correlations between first degree relatives than between second degree relatives; 3) Others had higher correlations between sibs than between parents and children. These familial patterns suggest that 1) the mutant NF1 allele, 2) unlinked modifying genes and 3) the normal NF1 allele may all be involved in the development of particular clinical features of NF1, but that their relative contributions vary for different features.